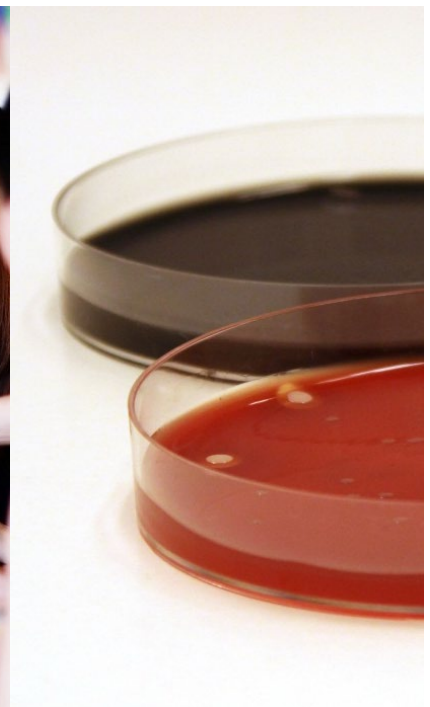


Food Microbiology

October 2021

Jonas Ilbäck



Edition

Version 1 (2021-12-13)

Editor in chief

Maria Sitell, head of the Department of Biology, Swedish Food Agency

Responsible for the scheme

Jonas Ilbäck, microbiologist, Department of Biology, Swedish Food Agency

PT October 2021 is registered as no. 2021/03076 at the Swedish Food Agency

Proficiency Testing
Microbiology – Food
October 2021

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
BcsA	<i>Bacillus cereus</i> selective agar
BEA	Bile esculin agar
BGLB	Brilliant green lactose bile broth
BP	Baird-Parker agar
Compact Dry EC	Compact Dry™ <i>E.coli</i> and coliforms
Compact Dry ETB	Compact Dry™ Enterobacteriaceae
Compact Dry ETC	Compact Dry™ Enterococcus
COMPASS	COMPASS® Enterococcus agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</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 Disk	3M™ Petrifilm™ Staph Express Disk
Petrifilm EB	3M™ Petrifilm™ Enterobacteriaceae
Petrifilm EC/CC	3M™ Petrifilm™ <i>E. coli</i> /Coliform Count
Petrifilm LAB	3M™ Petrifilm™ Lactic Acid Bacteria
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
RPFA	Baird-Parker agar with rabbit plasma fibrinogen
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® Coliform 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
IDF	International Dairy Foundation
ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
NordVal	NordVal International - NMKL
SLV	Livsmedelsverket/Swedish Food Agency, Sweden

Contents

General information on results evaluation.....	6
Results of the PT round October 2021	7
- General outcome	7
- Aerobic microorganisms, 30 °C and 20 °C	8
- Contaminating microorganisms in dairy products.....	11
- Enterobacteriaceae	12
- Coliform bacteria, 30 °C and 37 °C	14
- Thermotolerant coliform bacteria and <i>Escherichia coli</i>	17
- Presumptive <i>Bacillus cereus</i>	20
- Coagulase-positive staphylococci.....	22
- Enterococci	24
- Gram-negative bacteria in pasteurised milk and cream.....	26
Outcome of the results of individual laboratory – assessment	27
- Box plot	28
Test material and quality control	34
- Test material	34
- Quality control of the mixtures	35
References	36
Annex 1: Results obtained by the participants	
Annex 2: z-scores of all participants	

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 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 (PT) 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, in general 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
	results for all participants
	results deviating more than 1 s from m, or unusually many deviating results.

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

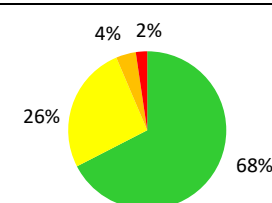
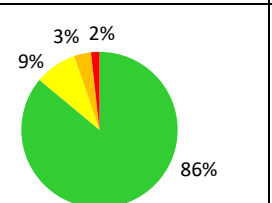
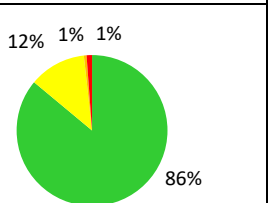
Results of the PT round October 2021

General outcome

Samples were sent to 182 laboratories, 46 in Sweden, 118 in other European countries, and 18 outside of Europe. Of the 172 laboratories that reported results, 77 (44 %) provided at least one result that received an annotation. In the previous round with similar analyses (October 2020) the proportion was 34 %.

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>Bacillus cereus</i> <i>Providencia alcalifaciens</i> <i>Staphylococcus aureus</i>				<i>Escherichia coli</i> <i>Enterococcus durans</i> <i>Serratia marcescens</i> <i>Staphylococcus aureus</i>				<i>Bacillus cereus</i> <i>Enterococcus faecium</i> <i>Staphylococcus xylosum</i>			
Analysis	Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Aerobic micro-organisms, 30 °C	All	162	0%	4%	All	161	0%	3%	All	161	0%	1%
Aerobic micro-organisms, 20 °C	All	24	4%	4%	All	24	0%	4%	All	24	0%	4%
Contaminating microorganisms	All	13	0%	0%	All	14	0%	0%	All	14	0%	0%
Enterobacteriaceae	<i>P. alcalifaciens</i>	145	3%	3%	<i>E. coli</i> <i>S. marcescens</i>	143	1%	3%	-	144	3%	0%
Coliform bacteria, 30 °C	(<i>P. alcalifaciens</i>)	40	18%	0%	<i>E. coli</i> (<i>S. marcescens</i>)	39	0%	0%	-	40	0%	0%
Coliform bacteria, 37 °C	(<i>P. alcalifaciens</i>)	88	18%	0%	<i>E. coli</i> (<i>S. marcescens</i>)	86	1%	1%	-	87	2%	0%
Thermotolerant coliform bacteria	-	45	2%	0%	<i>E. coli</i>	45	0%	2%	-	46	0%	0%
<i>Escherichia coli</i>	-	118	2%	0%	<i>E. coli</i>	115	2%	3%	-	117	1%	0%
Presumptive <i>B. cereus</i>	<i>B. cereus</i>	112	11%	1%	(<i>S. marcescens</i>) (<i>S. aureus</i>)	110	5%	0%	<i>B. cereus</i> (<i>S. xylosum</i>)	112	3%	4%
Coagulase-positive staphylococci	<i>S. aureus</i>	96	1%	11%	<i>S. aureus</i>	96	1%	6%	(<i>S. xylosum</i>)	98	5%	0%
Enterococci	-	60	2%	0%	<i>E. durans</i>	61	2%	3%	<i>E. faecium</i>	61	5%	10%
Gram-neg. bacteria in milk products	<i>P. alcalifaciens</i>	10	10%	-	<i>E. coli</i> <i>S. marcescens</i>	10	0%	-	-	10	0%	-

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

Aerobic microorganisms 30 °C and 20 °C

Sample A

The strains of *B. cereus*, *P. alcalifaciens* and *S. aureus* were target organisms for the analysis both at 20 °C and at 30 °C.

Sample B

The strains of *E. coli*, *E. durans*, *S. marcescens* and *S. aureus* were target organisms. *S. marcescens* and *S. aureus* were present in somewhat higher concentrations than *E. coli* and *E. durans*.

Sample C

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

General remarks

As in previous PT rounds most laboratories used either NMKL 86:2013, ISO 4833-1:2013 or 3M Petrifilm AC. Both NMKL 86:2013 and ISO 4833-1:2013 are based on incubation on PCA or MPCA at 30 °C for 72 h. Users of Petrifilm AC can use different times/temperatures, 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. ISO 4833-1:2013 was last reviewed by ISO in 2019 and remains current.

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.

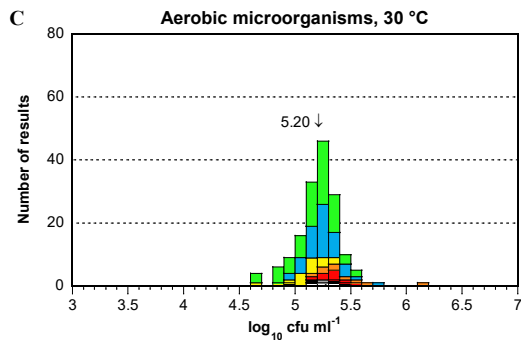
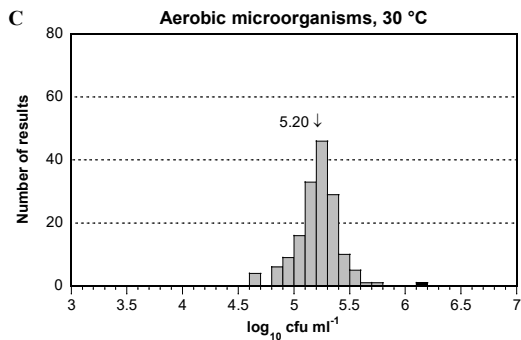
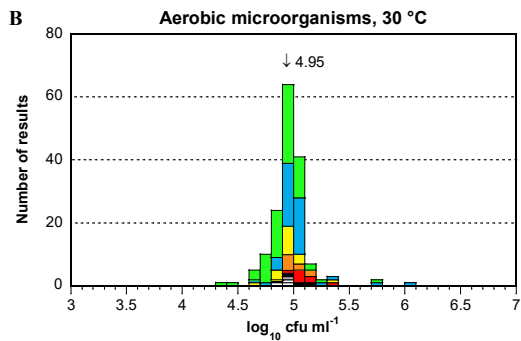
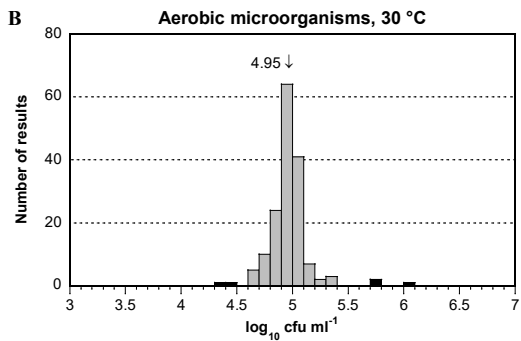
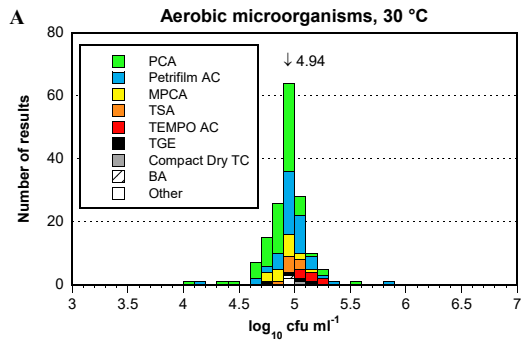
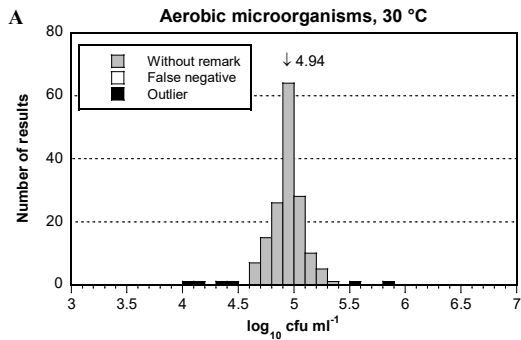
A few laboratories used TEMPO AC, which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains different-sized wells. A substrate in the medium emits fluorescence when hydrolysed by the microorganisms. The number of microorganisms is determined statistically by the number and size of the fluorescing wells. For the analysis at 30 °C, users of TEMPO AC reported somewhat higher results compared to other methods/media. A positive bias of this magnitude is often seen for TEMPO AC, and can be considered normal.

Comment: For the analysis at 30 °C, one laboratory followed ISO 13559/IDF 153 (contaminating microorganisms). However since the laboratory incubated on PCA, the results were still included in the evaluation. Also at 30 °C, one laboratory followed IDF 100B:1991. This method has been withdrawn, and replaced by ISO 4833.

Results from analysis of aerobic microorganisms, 30 °C

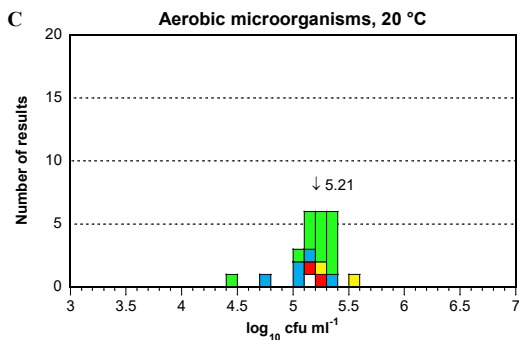
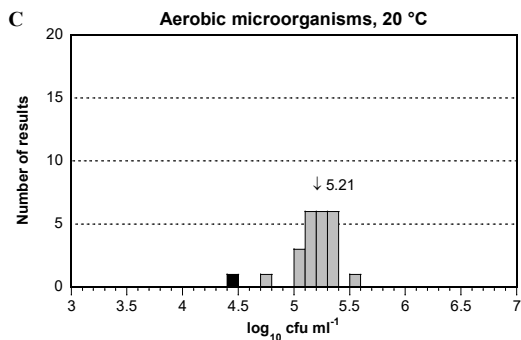
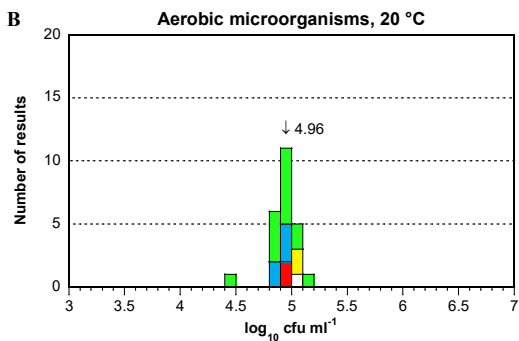
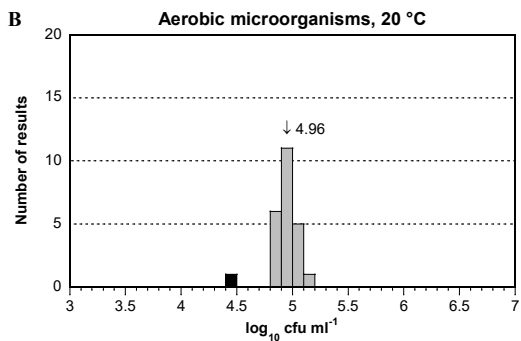
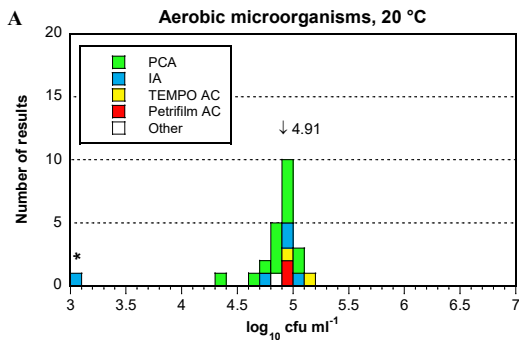
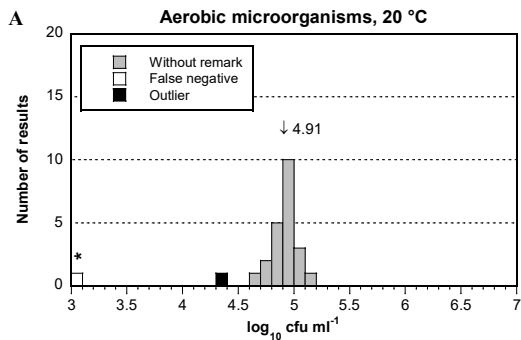
Medium	Sample A							Sample B							Sample C							
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>	
All results	162	156	4.94	0.13	0	4	2	161	156	4.95	0.12	0	2	3	161	160	5.20	0.18	0	0	0	1
PCA	71	67	4.89	0.13	0	3	1	71	68	4.91	0.12	0	2	1	71	71	5.16	0.19	0	0	0	0
Petrifilm AC ¹	49	47	4.97	0.12	0	1	1	48	46	4.97	0.10	0	0	2	48	48	5.23	0.15	0	0	0	0
MPCA	17	17	4.91	0.10	0	0	0	17	17	4.96	0.13	0	0	0	17	17	5.10	0.17	0	0	0	0
TSA	9	9	4.96	0.08	0	0	0	9	9	5.00	0.09	0	0	0	9	8	5.37	0.17	0	0	0	1
TEMPO AC	8	8	5.15	0.09	0	0	0	8	8	5.11	0.11	0	0	0	8	8	5.33	0.12	0	0	0	0
TGE	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0	0
Compact Dry TC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0	0
BA	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0	0
Other	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0	0

¹ Includes one laboratory that used Petrifilm RAC. All results for Petrifilm RAC were without remark.



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	24	22	4.91	0.11	1	1	0	24	23	4.96	0.08	0	1	0	24	23	5.21	0.16	0	1	0
PCA	14	13	4.89	0.11	0	1	0	14	13	4.96	0.08	0	1	0	14	13	5.25	0.11	0	1	0
IA	5	4	4.90	0.12	1	0	0	5	5	4.91	0.05	0	0	0	5	5	5.07	0.20	0	0	0
TEMPO AC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Petrifilm AC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Other	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0



Contaminating microorganisms

Sample A

The strains of *B. cereus*, *P. alcalifaciens* and *S. aureus* were target organisms. In the quality control at the Swedish Food Agency, three distinct colony morphologies were observed on medium without sugar (SFA). All of these were catalase-positive upon confirmation.

Sample B

The strains of *E. coli*, *E. durans*, *S. marcescens* and *S. aureus* can form colonies on SFA. The strain of *E. durans* is however catalase-negative and may therefore have been excluded during confirmation. At the same time, *E. durans* was present in such a low concentration in the sample, that exclusion of this strain should only have had a marginal effect on the result

Sample C

The strains of *B. cereus*, *E. faecium* and *S. xylosus* were target organisms. In the quality control at the Swedish Food Agency, three distinct colony morphologies were observed on SFA. *E. faecium* (small white colonies on SFA) is catalase-negative and laboratories may therefore have excluded it after confirmation.

General remarks

Only 14 laboratories performed the analysis and the results were therefore difficult to evaluate statistically. However when considering the species and concentrations of target organisms, the mean value of all laboratories, and the distribution of results that is normally seen in this analysis, no results were considered as outliers.

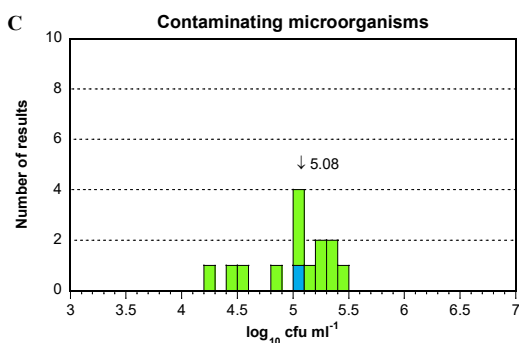
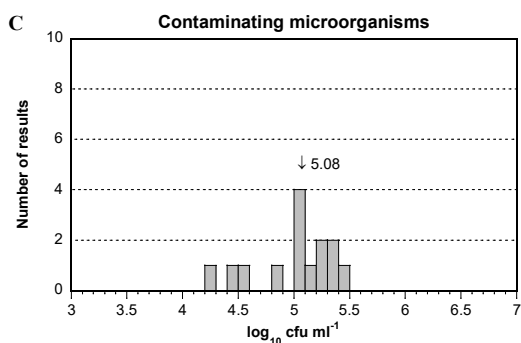
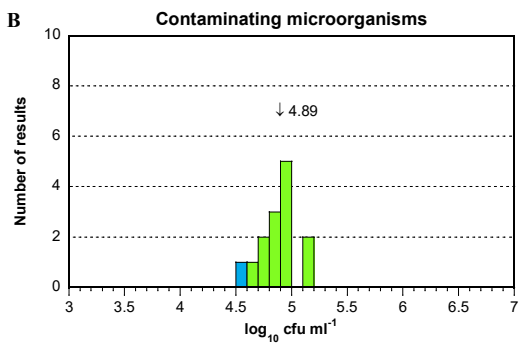
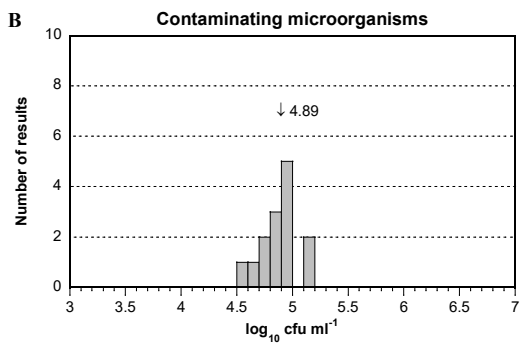
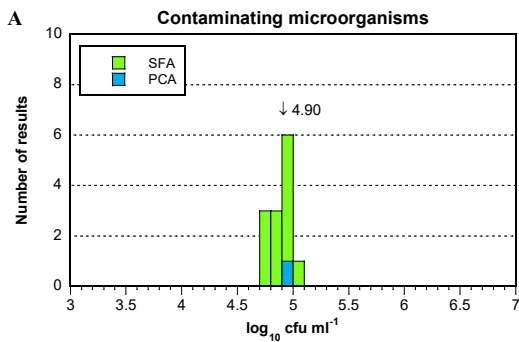
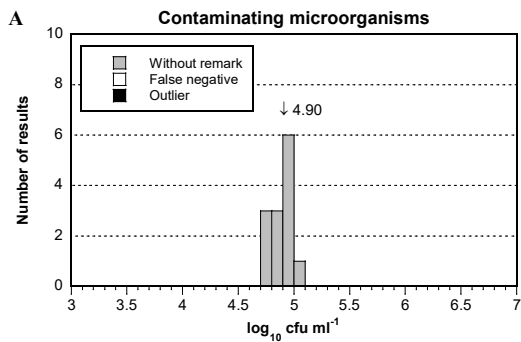
Eight of the 14 laboratories followed ISO 13559:2002 / IDF 153:2002. This was last reviewed by ISO in 2019 and remains current. One laboratory followed a modified version of the older IDF 153:1999. The remaining laboratories either followed internal methods, or did not specify further which method they used. All laboratories except one incubated on 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 14 laboratories stated performing a confirmation with a catalase test.

Results from analysis of contaminating microorganisms

Medium	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	4.90	0.10	0	0	0	14	14	4.89	0.17	0	0	0	14	14	5.08	0.37	0	0	0
SFA	12	12	4.90	0.10	0	0	0	13	13	4.90	0.15	0	0	0	13	13	5.08	0.38	0	0	0
PCA	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

* Med = median



Enterobacteriaceae

Sample A

The strain of *P. alcalifaciens* was target organism. It is oxidase-negative and forms typical colonies on VRBG.

Sample B

The strains of *E. coli* and *S. marcescens* belong to Enterobacteriaceae. Both are oxidase-negative and form red/purple colonies on VRBG. *S. marcescens* may form smaller colonies that are surrounded by a less prominent bile salt precipitation zone.

Sample C

No target organism was present in the sample. In the Swedish Food Agency's quality control, no colonies were observed on VRBG. Despite this, four laboratories reported a false positive result. Three of these were reported by laboratories that used Petrifilm EB.

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 PT rounds most laboratories followed either NMKL 144:2005 (39 %) or a method with Petrifilm EB (28 %), while the ISO methods (various versions) were used by 21 %. 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.

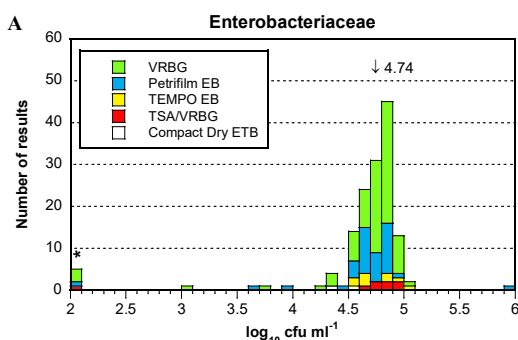
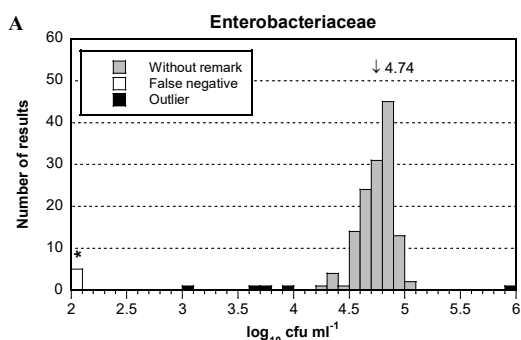
The number of users of ISO 21528-2:2017 was higher compared to ISO 21528-2:2004 (16 and 8 laboratories, respectively). In contrast, four laboratories stated the older ISO 21528-1:2004, while only three stated the new ISO 21528-1:2017. However, the reported results from the different ISO methods were similar.

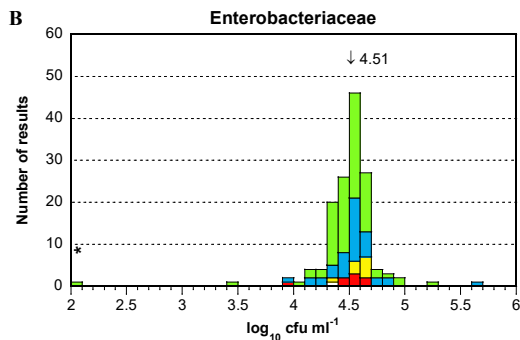
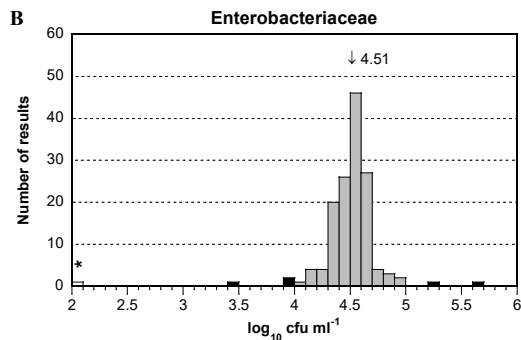
NMKL 144:2005 stipulates confirmation of presumptive colonies with an oxidase test. ISO 21528-2:2017 stipulates confirmation of presumptive colonies with both an oxidase test and with a test for glucose fermentation. Here, the majority of the laboratories that performed a confirmation test specified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

Overall, no apparent differences could be seen between the various methods, media and types of confirmation that were used by the laboratories.

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	145	135	4.74	0.15	5	4	1	143	137	4.51	0.15	1	3	2	144	140	-	-	4	-	-
VRBG	86	81	4.75	0.16	3	2	0	85	82	4.50	0.16	1	1	1	85	84	-	-	1	-	-
Petrifilm EB	40	36	4.72	0.12	1	2	1	40	38	4.51	0.16	0	1	1	40	37	-	-	3	-	-
TEMPO EB	9	9	4.76	0.19	0	0	0	9	9	4.59	0.09	0	0	0	9	9	-	-	0	-	-
TSA/VRBG	8	7	4.82	0.13	1	0	0	8	7	4.55	0.09	0	1	0	8	8	-	-	0	-	-
Compact Dry ETB	2	2	-	-	0	0	0	1	1	-	-	0	0	0	2	2	-	-	0	-	-





Coliform bacteria, 30 °C and 37 °C

Sample A

No target organism was present in the sample. The strain of *P. alcalifaciens* is however false positive for the analysis, and may form small colonies without a bile salt precipitation zone on VRB.

Seven false positive results were reported for the analysis at 30 °C and 16 false positive results were reported for the analysis at 37 °C.

The majority of the reported false-positive results were for concentrations around 4.7 log₁₀ cfu ml⁻¹, thus corresponding to the concentration of *P. alcalifaciens* in the sample. The false positive results could not be attributed to the use of a specific method or medium. False positive results were also reported both by laboratories that performed a confirmation and those that did not.

Sample B

The strain of *E. coli* was target organism. It forms red colonies with a precipitation zone on VRB. *S. marcescens* is a weak fermenter of lactose, and is capable of forming small colonies on VRB, with a less prominent precipitation zone. Both strains are oxidase-negative, but *S. marcescens* should be excluded after confirmation since, in contrast to *E. coli*, it does not produce gas in BGLB. *E. durans* and *S. aureus* are Gram-positive and should not form colonies on VRB.

The results at both 30 °C and 37 °C were distributed with two overlapping peaks, at approximately 4.0 and 4.5 log₁₀ cfu ml⁻¹. The two peaks could not be separated statistically, but the low and high peaks correspond very well to the concentrations of *E. coli* and of *E. coli* + *S. marcescens*, respectively.

It is difficult to attribute the low and high deviating results to the use of a specific method or medium. Methods and media used by only a few laboratories are hard to evaluate, but “low” and “high” results were reported at least by all of the major methods and media.

Sample C

No target organism was present in the sample. In the Swedish Food Agency's quality control, no colonies were observed on VRB. The only deviating results were two false positive results at 37 °C.

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.

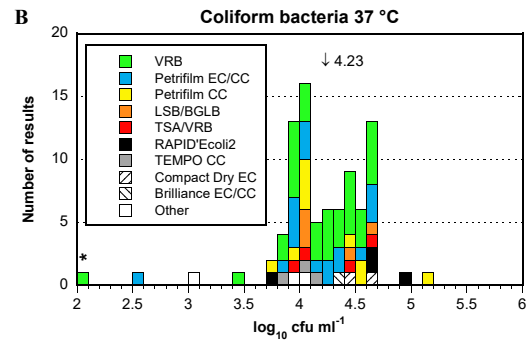
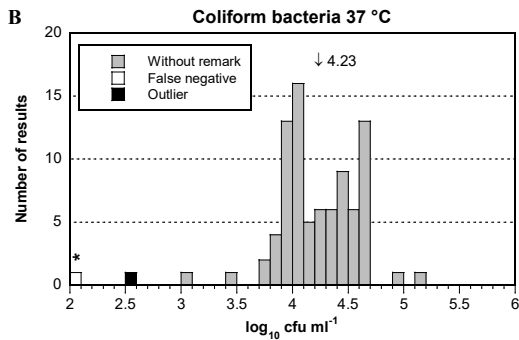
The most common methods were NMKL 44:2004 and ISO 4832:2006. At 37 °C, 3M™ Petrifilm™ was also used by many laboratories. Both NMKL 44:2004 and ISO 4832:2006 prescribe incubation on VRB, but the confirmation steps differ somewhat. With NMKL 44:2004 all presumptive colonies are confirmed. In contrast, with ISO 4832:2006 only atypical colonies require further confirmation. With Petrifilm CC, users are instructed that red colonies without gas bubbles should be picked and tested with “appropriate confirmation methods”. Differences in the definition of coliform bacteria—and the interpretation of “typical colonies” by the laboratories—may be part of the explanation for the deviating results in samples A and B. ISO 4832:2006 was last reviewed by ISO in 2021, and remains current.

LSB in combination with BGLB was used by laboratories that followed the MPN-based methods ISO 4831:2006 and NMKL 96:2009. ISO 4831:2006 is adapted for use when the expected concentration of coliform bacteria is lower than or equal to 100 cfu g⁻¹. It was last reviewed by ISO in 2021 and remains current. NMKL 96:2009 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 PT rounds, users of these methods have reported somewhat deviating results, likely since they are not adapted for the concentrations in the PT samples. However this does not appear to have been the case this time.

Some methods and media were used only by a few laboratories, but should still be mentioned. These included 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 other media that use a similar principle for detecting and distinguishing coliform bacteria and *E. coli* are Compact Dry EC and Brilliance EC/CC. Though the low number of users makes these media difficult to evaluate, it can still be noted that no deviating results were reported by the laboratories that used them. Finally, a few laboratories performed a pre-incubation on TSA, which is recommended by some methods if the sample is suspected to contain stressed coliform bacteria.

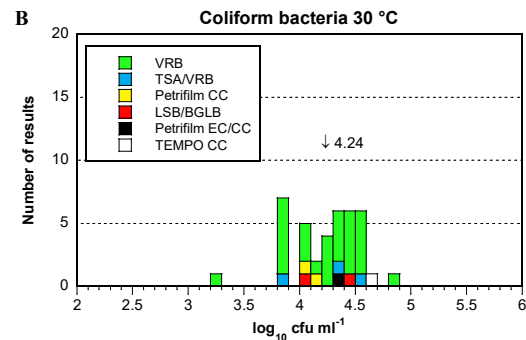
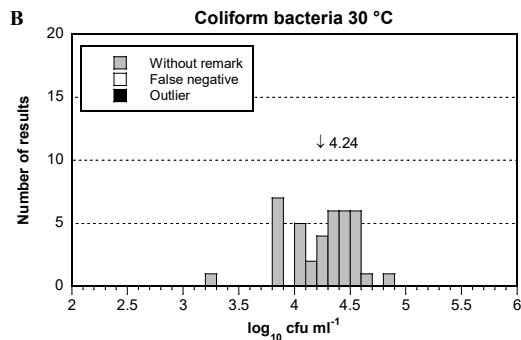
Results from analysis of coliform bacteria, 37 °C

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	88	72	-	-	16	- -	86	84	4.23	0.33	1	1	0	87	85	-	-	2	- -
VRB	38	30	-	-	8	- -	36	35	4.23	0.28	1	0	0	38	36	-	-	2	- -
Petrifilm EC/CC	18	13	-	-	5	- -	18	17	4.20	0.28	0	1	0	18	18	-	-	0	- -
Petrifilm CC	10	8	-	-	2	- -	10	10	4.26	0.40	0	0	0	9	9	-	-	0	- -
LSB/BGLB	5	4	-	-	1	- -	5	5	4.25	0.29	0	0	0	5	5	-	-	0	- -
TSA/VRB	4	4	-	-	0	- -	4	4	-	-	0	0	0	4	4	-	-	0	- -
RAPID' E.coli2	4	4	-	-	0	- -	4	4	-	-	0	0	0	4	4	-	-	0	- -
TEMPO CC	3	3	-	-	0	- -	3	3	-	-	0	0	0	3	3	-	-	0	- -
Compact Dry EC	2	2	-	-	0	- -	2	2	-	-	0	0	0	2	2	-	-	0	- -
Brilliance EC/CC	1	1	-	-	0	- -	1	1	-	-	0	0	0	1	1	-	-	0	- -
Other	3	3	-	-	0	- -	3	3	-	-	0	0	0	3	3	-	-	0	- -



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	40	33	-	-	7	- -	39	39	4.24	0.31	0	0	0	40	40	-	-	0	- -
VRB	31	25	-	-	6	- -	30	30	4.23	0.32	0	0	0	31	31	-	-	0	- -
TSA/VRB	3	3	-	-	0	- -	3	3	-	-	0	0	0	3	3	-	-	0	- -
Petrifilm CC	2	1	-	-	1	- -	2	2	-	-	0	0	0	2	2	-	-	0	- -
LSB/BGLB	2	2	-	-	0	- -	2	2	-	-	0	0	0	2	2	-	-	0	- -
Petrifilm EC/CC	1	1	-	-	0	- -	1	1	-	-	0	0	0	1	1	-	-	0	- -
TEMPO CC	1	1	-	-	0	- -	1	1	-	-	0	0	0	1	1	-	-	0	- -



Thermotolerant coliform bacteria and *Escherichia coli*

Sample A

No target organism was present in sample C, neither for the analysis of thermotolerant coliform bacteria nor for *E. coli*. In the Swedish Food Agency's quality control, no colonies were observed on VRB.

Sample B

The strain of *E. coli* was target organism for both analyses. At 44 °C, it forms typical red colonies surrounded by a bile salt precipitation zone on VRB. The strain produces gas and indole in LTLNB and it is positive for β-glucuronidase.

Sample C

No target organism was present in the sample, neither for the analysis of thermotolerant coliform bacteria nor for *E. coli*. In the Swedish Food Agency's quality control, no colonies were observed on VRB.

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 bile salt precipitation. Thermotolerant coliform bacteria also produce gas as a consequence of lactose fermentation. *E. coli* can be distinguished from other thermotolerant coliform bacteria by confirmation, since they also produce indole, and since they possess the enzyme β-glucuronidase.

NMKL 125:2005 was the most commonly used method for the analysis of thermotolerant coliform bacteria (58 % 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 therefore *E. coli* form blue-green colonies on these media. The plastic film in Petrifilm EC/CC and Petrifilm SEC also enables 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. ISO 16649-2:2001 was last reviewed by ISO in 2019 and remains

current. NMKL 125 is currently being revised, and the new version will likely be more similar to ISO 16649-2.

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*. It was last reviewed by ISO in 2019 and remains current. 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 typically incubated in LSB/EC. Users of LSB/EC—and also users of TEMPO EC—reported slightly higher results compared to other methods/media. This has been seen in several previous PT rounds, and can therefore be considered normal.

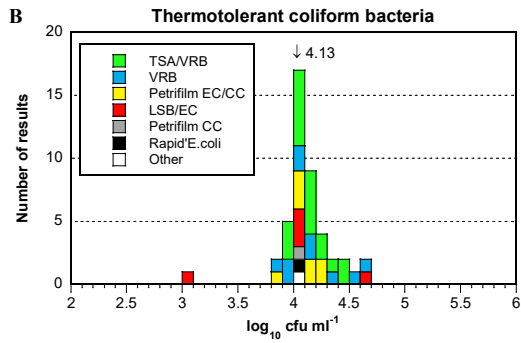
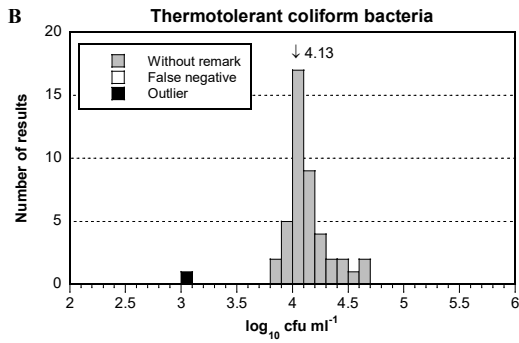
For *E. coli*, laboratories may occasionally get somewhat lower results with TBX, and somewhat higher results with TSA/VRB, compared to other media. Such differences can be due to performing, or not performing, a pre-incubation at a lower temperature. Here, the mean values for TSA/VRB and TBX did not deviate significantly from other media, and the results were within one standard deviation from the mean value of all results.

For *E. coli*, incubation was normally done either at 42-44 °C or at 35-37 °C, depending on which method was used. The mean values of the two temperature groups did not differ significantly, 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, 88 % of the laboratories that followed NMKL 125:2005 for the analysis of *E. coli* performed a confirmation test. 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	45	44	-	-	1	-	-	45	44	4.13	0.19	0	1	0	46	46	-	-	0	-	-
TSA/VRB	19	19	-	-	0	-	-	19	19	4.14	0.15	0	0	0	19	19	-	-	0	-	-
VRB	10	10	-	-	0	-	-	10	10	4.16	0.27	0	0	0	11	11	-	-	0	-	-
Petrifilm EC/CC	8	7	-	-	1	-	-	8	8	4.09	0.13	0	0	0	8	8	-	-	0	-	-
LSB/EC ¹	5	5	-	-	0	-	-	5	4	-	-	0	1	0	5	5	-	-	0	-	-
Petrifilm CC	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
Rapid [®] E.coli	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
Other	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-

¹ Represents laboratories that used MPN-based methods, where EC is normally used after a first enrichment in LSB.



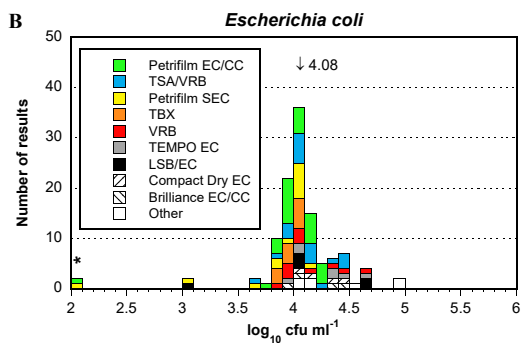
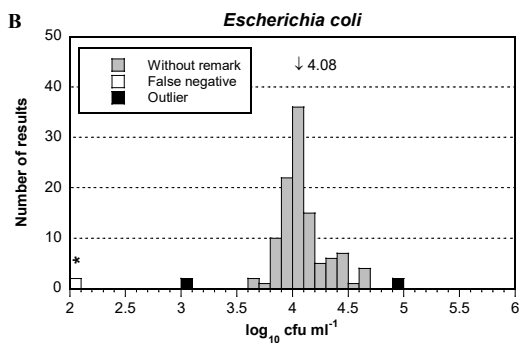
Results from analysis of *Escherichia coli*

Medium	Sample A						Sample B						Sample C					
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >
All results	118	116	-	-	2	- -	115	109	4.08	0.21	2	2 2	117	116	-	-	1	- -
Petrifilm EC/CC	29	27	-	-	2	- -	29	28	4.02	0.14	1	0 0	29	29	-	-	0	- -
TSA/VRB ¹	20	20	-	-	0	- -	20	20	4.10	0.20	0	0 0	20	20	-	-	0	- -
Petrifilm SEC	15	15	-	-	0	- -	14	12	3.98	0.15	1	1 0	14	13	-	-	1	- -
TBX	14	14	-	-	0	- -	13	13	3.97	0.10	0	0 0	14	14	-	-	0	- -
VRB	11	11	-	-	0	- -	11	11	4.11	0.26	0	0 0	11	11	-	-	0	- -
TEMPO EC	7	7	-	-	0	- -	7	7	4.25	0.26	0	0 0	7	7	-	-	0	- -
LSB/EC ²	6	6	-	-	0	- -	6	5	4.29	0.34	0	1 0	6	6	-	-	0	- -
Compact Dry EC	4	4	-	-	0	- -	4	4	-	-	0	0 0	4	4	-	-	0	- -
Brilliance EC/CC	4	4	-	-	0	- -	4	4	-	-	0	0 0	4	4	-	-	0	- -
Other ³	8	8	-	-	0	- -	7	5	-	-	0	0 2	8	8	-	-	0	- -

¹ Includes two laboratories that used TSA/VRBG.

² Represents laboratories that used MPN-based methods, where EC is normally used after a first enrichment in LSB.

³ Includes e.g. Rapid'E.coli 2 Petrifilm REC and Rebecca agar. Also includes one laboratory that used MPN-based Colilert QuantiTray 18 h (Idexx), which is used for detection of coliform bacteria and *E. coli* in water.



Presumptive *Bacillus cereus*

Sample A

The strain of *B. cereus* was target organism for the analysis. On BA it forms typical grey colonies that are surrounded by a distinct zone of haemolysis. On BcsA it forms typical blue colonies surrounded by a blue zone of precipitation. The strains of *P. alcalifaciens* and *S. aureus* may also form colonies on BA; they are however easily separated from *B. cereus* based on their appearance on BA and BcsA.

Sample B

No target organism for the analysis was present in the sample. Laboratories may mistakenly have included either *S. marcescens* or *S. aureus*. During the initial quality control of the sample at the Swedish Food Agency, small atypical colonies were observed on BA. Upon confirmation, they formed atypical colonies without blue colouring on BcsA.

Sample C

A strain of *B. cereus*—not identical to the one in sample A—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.

General remarks

Most laboratories followed either NMKL 67:2010 (54 %) or ISO 7932:2004 (22 %), which differ somewhat. NMKL 67:2010 is based on primary incubation on BA, and colonies are confirmed on a selective medium, e.g. BcsA. In comparison, ISO 7932:2004 prescribes incubation on MYP, which is followed by confirmation on BA. An amendment is available for the ISO method (ISO 7932:2004/Amd 1:2020). It contains optional tests, including for PCR detection of *cytK* genes. A new version of NMKL 67 is also available; NMKL 67:2021. This version is more similar to ISO 7932:2004 in that BcsA is used as the primary plating medium, and BA as the medium for confirmation. In this PT, three laboratories had already adopted the new NMKL 67:2021.

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 MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity.

As in previous PT 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 similar, with the exception of the two instances mentioned below.

Compact Dry X-BC was used by seven laboratories. The chromogenic and selective agents in this medium cause *B. cereus* to form blue/green colonies, whereas other bacteria normally form white colonies. For sample A, two false negative results were reported by users of this method. The mean value for the remaining five results was

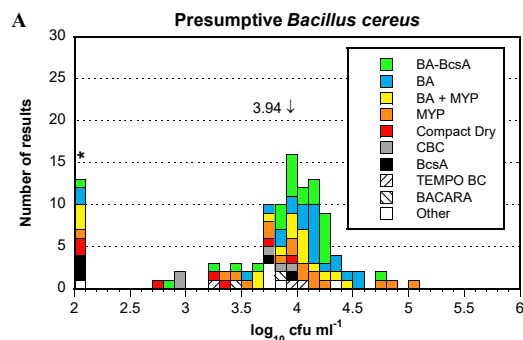
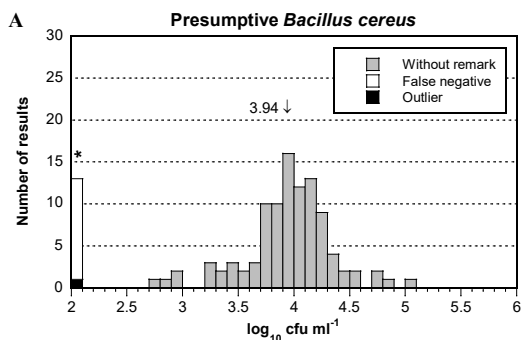
approximately 0.5 log₁₀ cfu/ml lower than the mean value for all methods. A slight negative bias of a similar magnitude is not unexpected for Compact Dry X-BC compared to the reference method ISO 7932:2004; this is also mentioned in both the NordVal 045 and MicroVal 2011-LR41 validations. It has also been seen in previous PT rounds by the Swedish Food Agency, for example when the same strain of *B. cereus* was included in PT Food April 2021. Slightly lower results for Compact Dry X-BC can therefore be considered normal. False negative results should however raise concern for the affected laboratories.

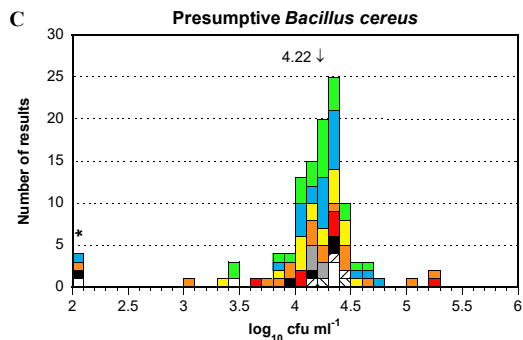
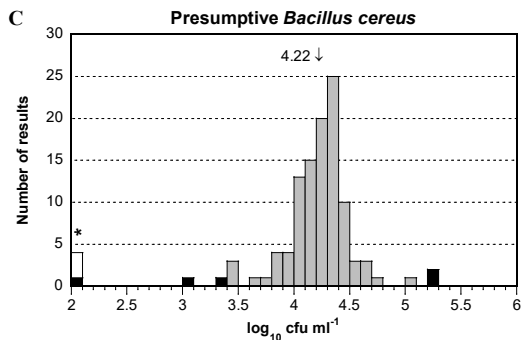
The chromogenic medium CBC was used by five laboratories. CBC contains the substrate X-Gluc, which is cleaved by *B. cereus* β-glucuronidase. Colonies of *B. cereus* are therefore white with a blue/green centre on this medium. Similar to Compact Dry X-BC, the mean value for CBC was somewhat lower than the mean value for all methods. However in contrast to Compact Dry X-BC, this has not been seen previously for CBC in the PT organised by the Swedish Food Agency. It could therefore simply be a random occurrence.

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	112	99	3.94	0.39	12	1	0	110	105	-	-	5	-	-	112	104	4.22	0.24	3	3	2
BA + BcsA	25	24	3.97	0.38	1	0	0	25	25	-	-	0	-	-	25	25	4.17	0.28	0	0	0
BA	24	22	4.10	0.24	2	0	0	23	20	-	-	3	-	-	24	23	4.26	0.19	1	0	0
BA + MYP	18	15	4.00	0.22	2	1	0	18	18	-	-	0	-	-	18	17	4.23	0.19	0	1	0
MYP	17	16	4.03	0.49	1	0	0	16	14	-	-	2	-	-	18	15	4.25	0.32	1	1	1
Compact Dry X-BC	7	5	3.39	0.45	2	0	0	7	7	-	-	0	-	-	7	6	4.11	0.29	0	0	1
CBC	5	5	3.48	0.52	0	0	0	5	5	-	-	0	-	-	5	5	4.20	0.06	0	0	0
BcsA	5	2	-	-	3	0	0	5	5	-	-	0	-	-	5	4	-	-	1	0	0
TEMPO BC	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
BACARA™	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
Other ¹	6	5	-	-	1	0	0	6	6	-	-	0	-	-	5	4	-	-	0	1	0

¹ Includes COMPASS® *Bacillus cereus* agar, Brilliance™ *Bacillus cereus* and PEMBA.





Coagulase-positive staphylococci

Sample A

The coagulase-positive strain of *S. aureus* was target organism for the analysis. On RPFA, it forms typical convex grey/black colonies surrounded by a precipitation zone.

Sample B

A strain of *S. aureus*—not identical to the one in sample A—was target organism. On RPFA, it forms typical convex grey/black colonies surrounded by a precipitation zone.

Sample C

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 precipitation zone. The strain may also form grey colonies on BP.

General remarks

Most laboratories (43 %) followed NMKL 66:2009. Other major methods were 3M™ Petrifilm™ (18 %), ISO 6888-1:1999 (13 %) and ISO 6888-2:1999 (7 %). Both of the ISO methods are now withdrawn and replaced by ISO 6888-1:2021 and ISO 6888-2:2021, respectively. One laboratory 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. In comparison, ISO 6888-1:2021 stipulates surface spreading on BP, whereas 6888-2:2021 stipulates the use of RPFA. On BP, *S. aureus* form 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. With BP, colonies are usually confirmed by a positive result in a coagulase test. With RPFA, the coagulase activity is instead tested directly in the medium. 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.

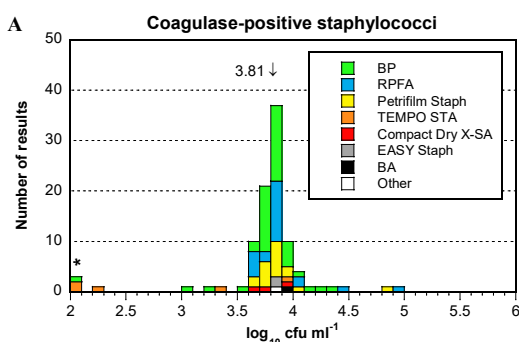
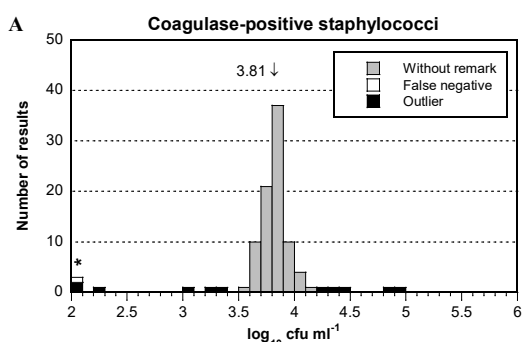
Taken together, the results were very similar for the most common media BP, RPFA and Petrifilm Staph, in all three samples. Outliers and false results could not be attributed to the use of a specific method, medium or confirmation test. Somewhat

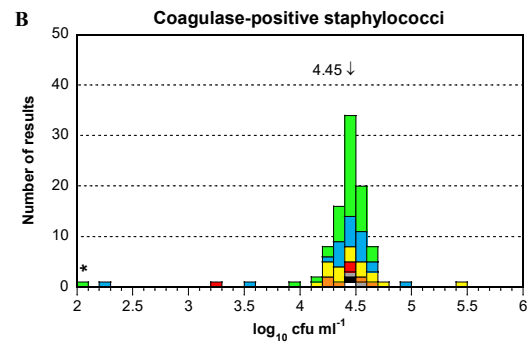
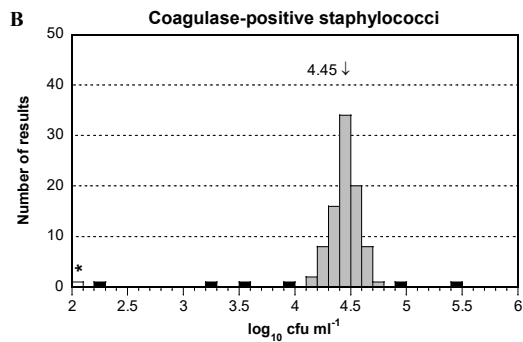
lower mean values have in previous PT rounds sometimes been seen for Petrifilm Staph, but this was not evident this time. TEMPO STA, Compact Dry™ X-SA and EASY Staph®, and Brilliance™ Staph 24 were only used by a small number of laboratories, which makes them difficult to evaluate. One laboratory used BA, in combination with StafChrom.

In total, 72 % 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 almost exclusively 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	96	84	3.81	0.10	1	6 5	96	89	4.45	0.11	1	4 2	98	93	-	-	5	-	-
BP	43	38	3.81	0.11	1	2 2	44	42	4.45	0.10	1	1 0	45	44	-	-	1	-	-
RPFA	23	21	3.81	0.11	0	0 2	23	20	4.45	0.11	0	2 1	23	22	-	-	1	-	-
Petrifilm Staph	18	17	3.82	0.10	0	0 1	17	16	4.45	0.16	0	0 1	18	16	-	-	2	-	-
TEMPO STA	5	1	-	-	0	4 0	5	5	4.43	0.16	0	0 0	5	5	-	-	0	-	-
Compact Dry X-SA	3	3	-	-	0	0 0	3	2	-	-	0	1 0	3	2	-	-	1	-	-
EASY Staph	2	2	-	-	0	0 0	2	2	-	-	0	0 0	2	2	-	-	0	-	-
BA	1	1	-	-	0	0 0	1	1	-	-	0	0 0	1	1	-	-	0	-	-
Other	1	1	-	-	0	0 0	1	1	-	-	0	0 0	1	1	-	-	0	-	-





Enterococci

Sample A

No target organism was present in the sample. In the Swedish Food Agency's quality control on Slanetz & Bartley *Enterococcus* agar (ENT), no colonies were observed on the plates.

Sample B

The strain of *E. durans* was target organism. On ENT it forms typical raised, dark red colonies. Upon confirmation on BEA a faint tan/black colour is normally seen in the medium after 2 hours, and a distinct black colour after 24 hours.

Sample C

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.

General remarks

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-trifenylyltetrazolium 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.

A clear majority of the laboratories (63 %) followed NMKL 68:2011. Among the less frequently used methods were the drinking water method ISO 7899-2:2000 (8 %) and IDF 149A:1997 (7 %). The older NMKL 68:2004 was used by two laboratories. 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 2021 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

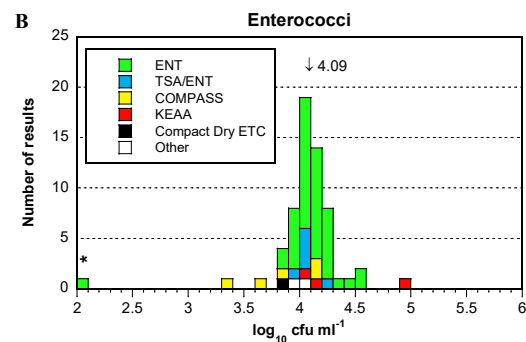
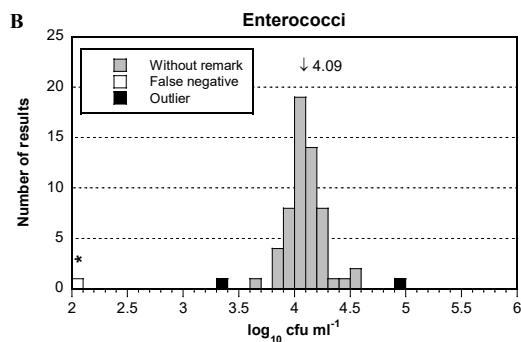
7899-2:2000 is based on membrane filtration and incubation is on ENT at 37 °C. Confirmation is done similarly to the NMKL method, but by transfer of the entire membrane filter from ENT to BEA (possibly with the addition of azide).

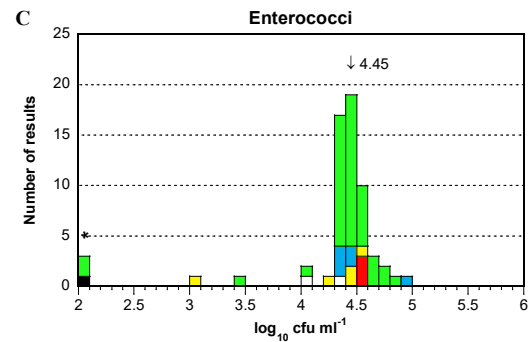
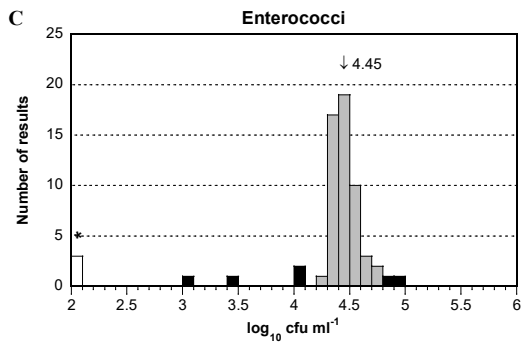
In total, 82 % of the laboratories incubated either on ENT or on TSA/ENT. A smaller number of laboratories used COMPASS® Enterococcus agar, KEAA or Compact Dry ETC. KEAA was used by laboratories that followed IDF 149A:1997. With KEAA, hydrolysis of esculin is detected directly in this medium. Similar 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, 79 % of the laboratories stated that they performed some kind of confirmation.

The laboratories performed the primary incubation either at 44 °C (75 %) or at 37 °C (25 %). Previously, there has been a suspicion that low results for the strain in sample C is associated with incubation at 37 °C. Such an association was however not evident this time, and the few low outliers for sample C could also be the result of for example a short incubation time on BEA in the confirmation step.

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	60	59	-	-	1	-	-	61	58	4.09	0.16	1	1	1	61	52	4.45	0.10	3	4	2
ENT	43	43	-	-	0	-	-	44	43	4.11	0.16	1	0	0	44	39	4.45	0.11	2	2	1
TSA/ENT	6	6	-	-	0	-	-	6	6	4.06	0.13	0	0	0	6	5	4.38	0.03	0	0	1
COMPASS®	5	5	-	-	0	-	-	5	4	-	-	0	1	0	5	4	-	-	0	1	0
KEAA	3	3	-	-	0	-	-	3	2	-	-	0	0	1	3	3	-	-	0	0	0
Compact Dry ETC	1	0	-	-	1	-	-	1	1	-	-	0	0	0	1	0	-	-	1	0	0
Other	2	2	-	-	0	-	-	2	2	-	-	0	0	0	2	1	-	-	0	1	0





Gram-negative bacteria in pasteurised milk and cream

Sample A

The strain of *P. alcalifaciens* is Gram-negative.

Sample B

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

Sample C

No target organism was present in the sample.

General remarks

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.

Eight of the ten laboratories followed NMKL 192:2011. The two remaining laboratories followed a company-specific method. Nine of ten laboratories incubated on VRBG, while one used MacConkey agar.

All reported results were correct, except for one false negative result for sample A.

Results from analysis of Gram-negative bacteria in pasteurised milk and cream

Method	Sample A			Sample B			Sample C		
	N	n	F	N	n	F	N	n	F
All results	10	10	1	10	10	0	10	10	0
NMKL 192:2011	8	7	1	8	8	0	8	8	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 reporting results for the wrong sample, the results cannot be correctly processed. Incorrectly reported results are as a general rule excluded but may—after manual assessment by the Swedish Food Agency in each individual case—still be included and processed.

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

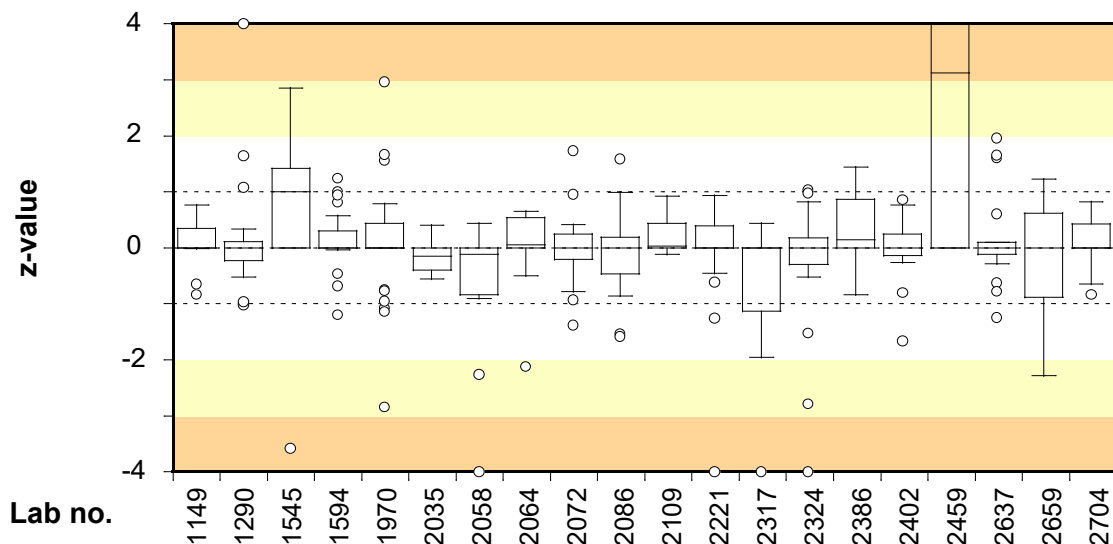
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated 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. 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. A small range of values, 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. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

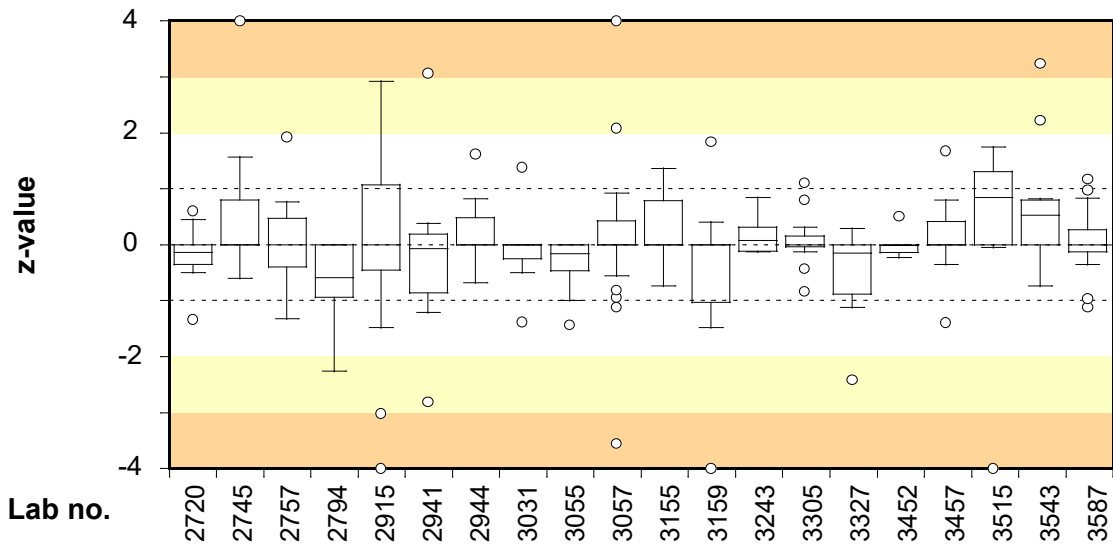
Box plots and numbers of deviating results for each laboratory

- 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.

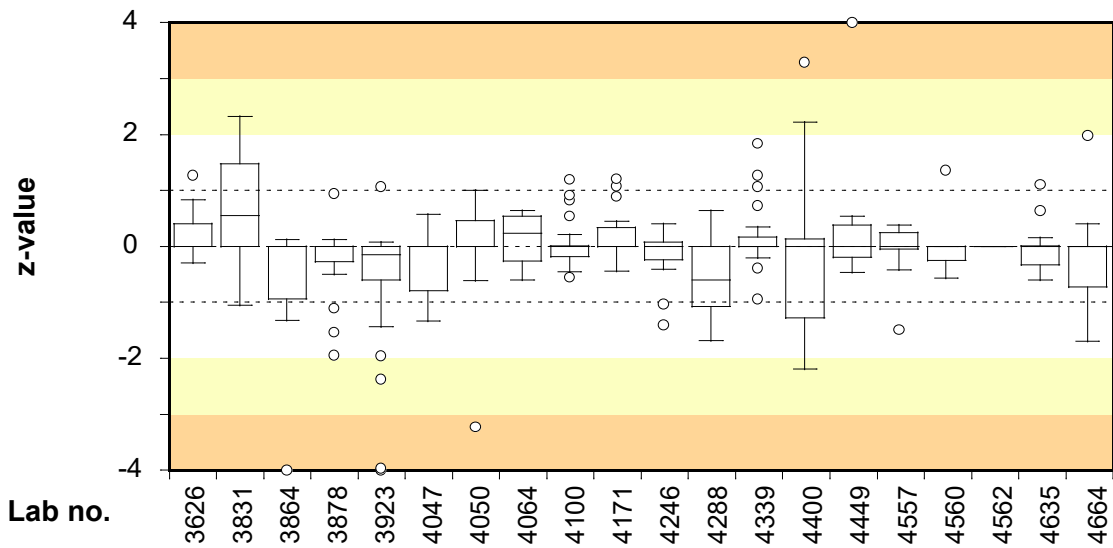
* $< [\text{lowest value in the box} - 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$
or
 $> [\text{highest value in the box} + 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$.



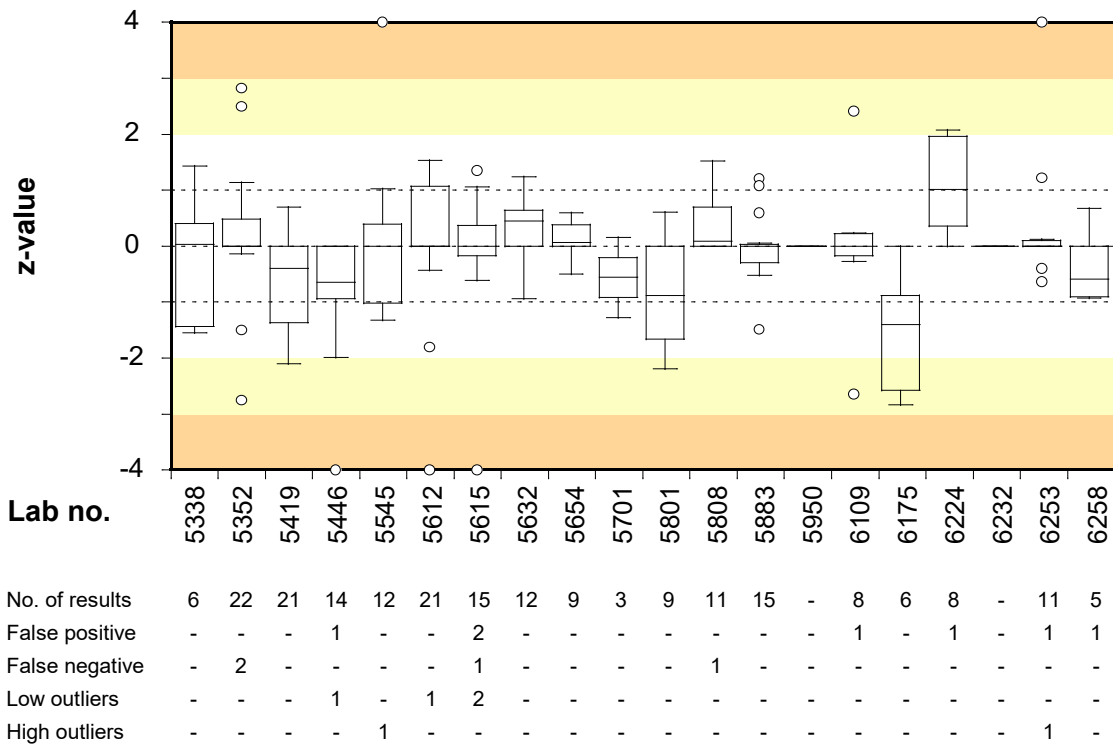
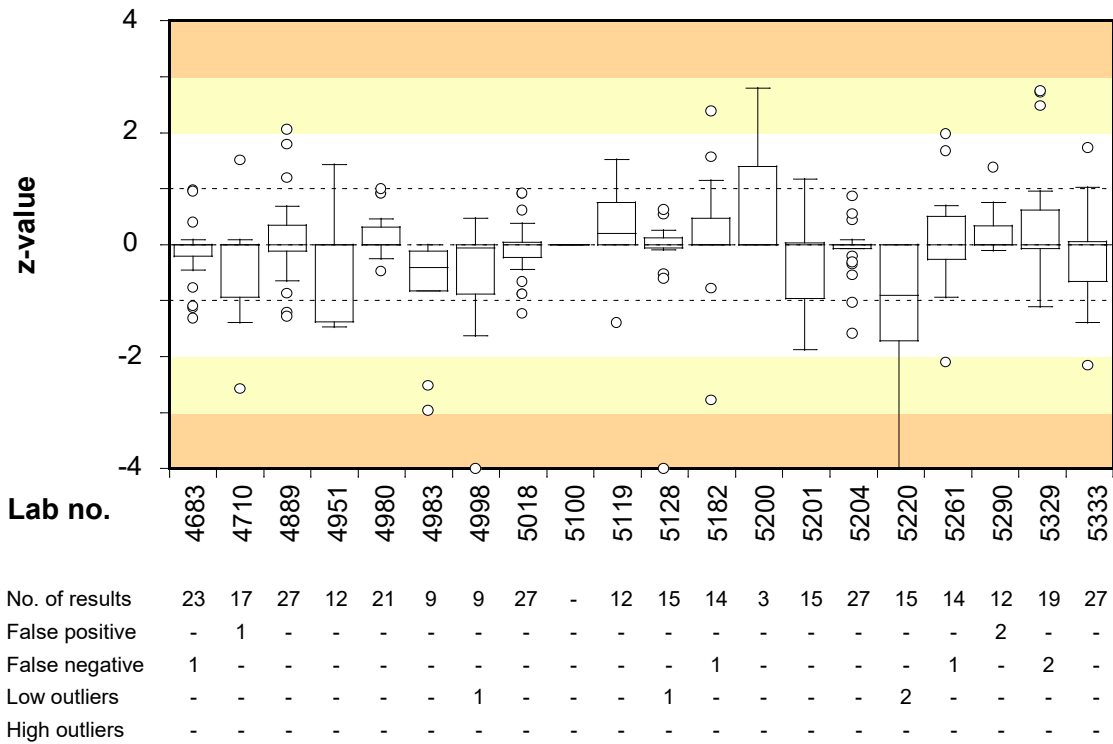
Lab no.	1149	1290	1545	1594	1970	2035	2058	2064	2072	2086	2109	2221	2317	2324	2386	2402	2459	2637	2659	2704
No. of results	15	17	21	26	29	6	11	9	30	20	6	25	21	21	18	11	17	18	8	18
False positive	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-	1	1	-	-	-
False negative	-	1	-	-	1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	1	-	-	-	1	-	-	-	-	1	1	2	-	-	-	-	-	-
High outliers	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-

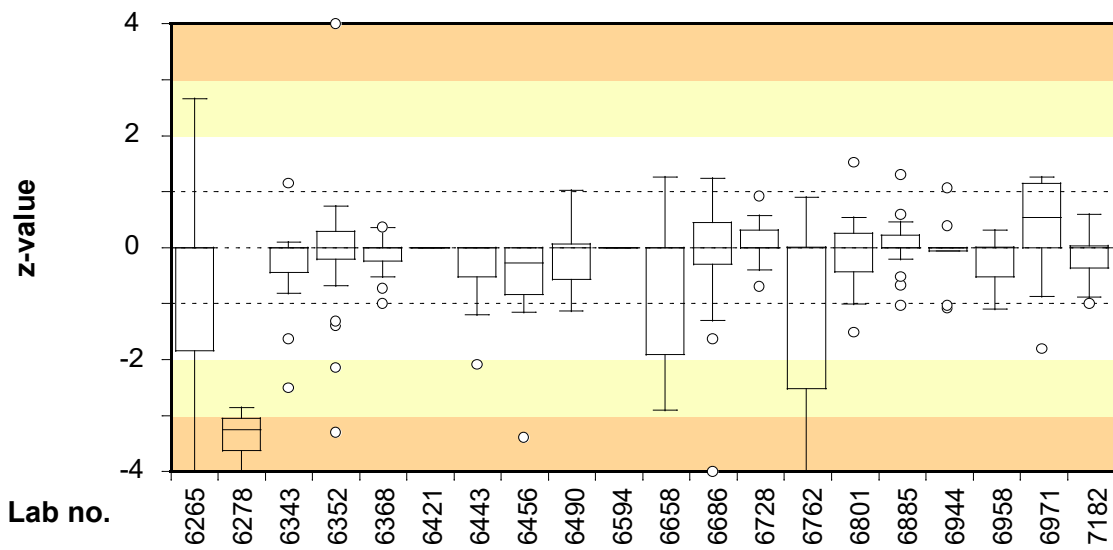


No. of results	9	18	11	6	21	8	18	7	12	27	17	18	6	18	12	5	24	15	14	24	
False positive	-	-	1	-	-	5	-	1	-	-	1	-	-	-	-	-	1	-	-	-	-
False negative	-	-	-	-	-	5	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-
Low outliers	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-
High outliers	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-

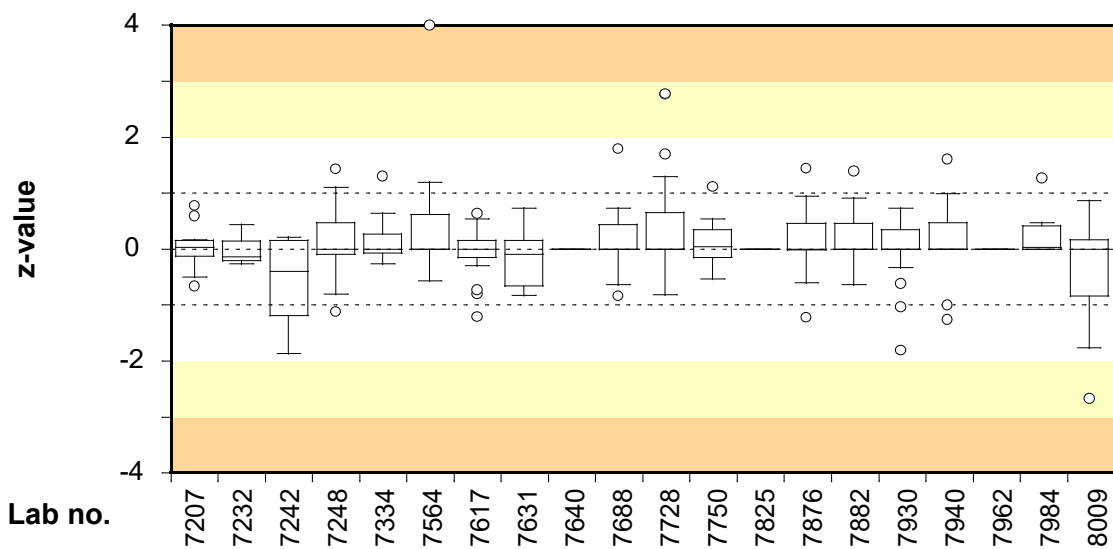


No. of results	27	12	9	17	27	15	18	6	27	18	12	21	30	9	11	15	12	-	14	21	
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
False negative	-	-	-	1	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-
Low outliers	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-

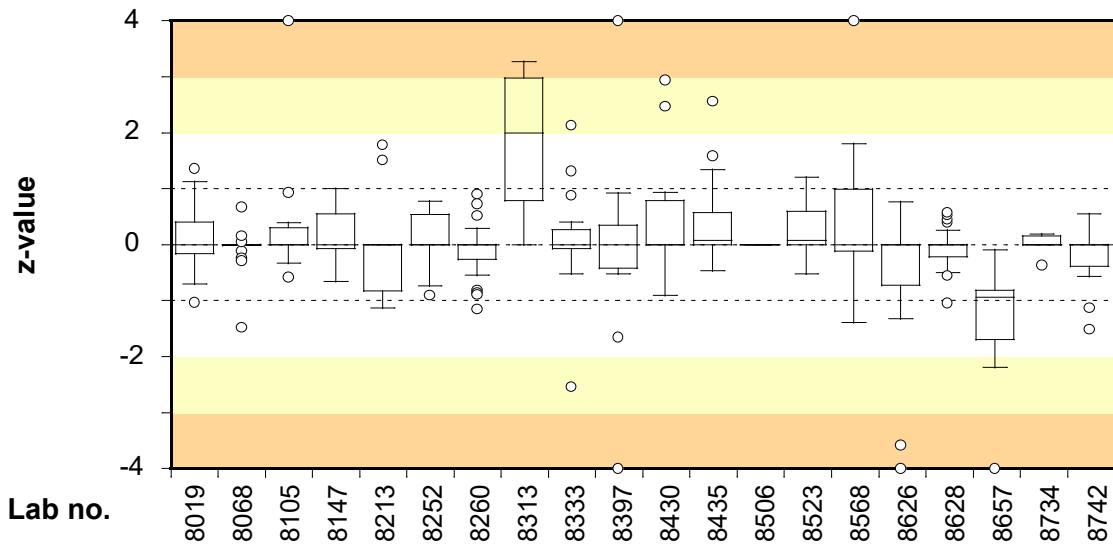




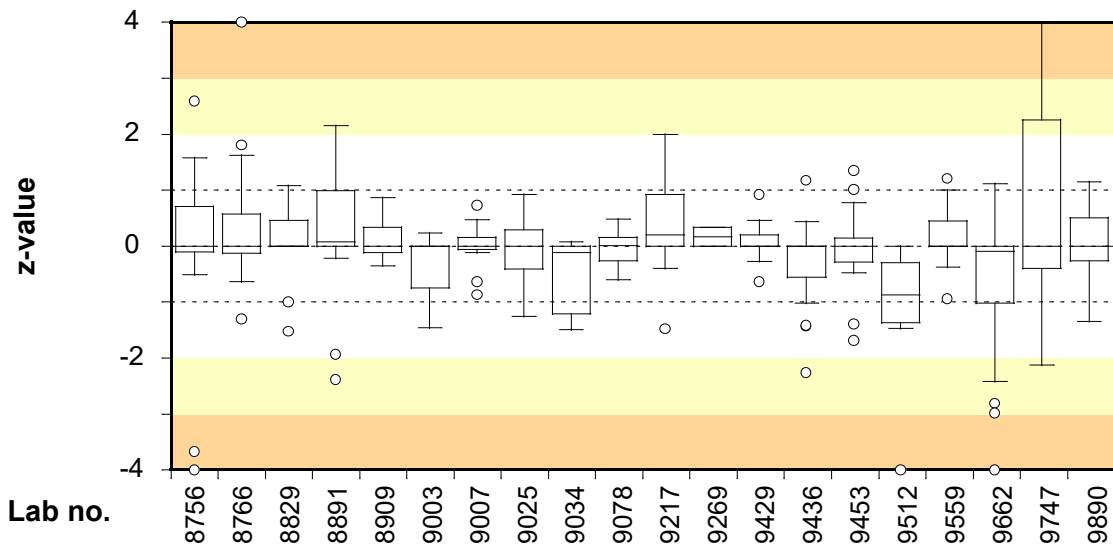
Lab no.	6265	6278	6343	6352	6368	6421	6443	6456	6490	6594	6658	6686	6728	6762	6801	6885	6944	6958	6971	7182
No. of results	33	3	16	21	27	-	9	13	18	-	12	20	14	8	12	21	9	9	9	12
False positive	1	-	1	-	-	-	-	2	-	-	-	1	1	1	-	-	-	-	-	-
False negative	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	7	1	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-	-
High outliers	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



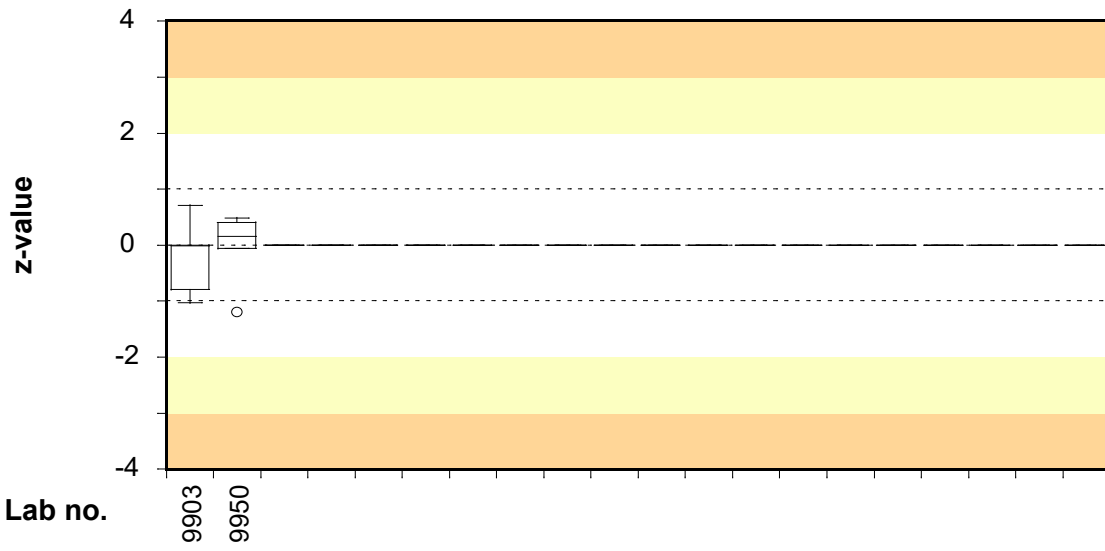
Lab no.	7207	7232	7242	7248	7334	7564	7617	7631	7640	7688	7728	7750	7825	7876	7882	7930	7940	7962	7984	8009
No. of results	12	3	6	33	12	18	15	8	-	27	24	8	-	18	17	21	9	-	12	10
False positive	-	-	-	-	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	8019	8068	8105	8147	8213	8252	8260	8313	8333	8397	8430	8435	8506	8523	8568	8626	8628	8657	8734	8742
No. of results	29	18	15	12	12	18	27	12	15	17	15	30	-	12	15	15	30	7	6	15
False positive	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	2	-	1	-	-
High outliers	-	-	1	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-



Lab no.	8756	8766	8829	8891	8909	9003	9007	9025	9034	9078	9217	9269	9429	9436	9453	9512	9559	9662	9747	9890
No. of results	18	18	15	20	18	15	12	12	12	6	14	2	18	27	18	8	22	26	8	19
False positive	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	-	2
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-
Low outliers	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-
High outliers	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-



No. of results	18	6
False positive	-	-
False negative	-	-
Low outliers	-	-
High outliers	-	-

Test material and quality control

Test material

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

Table 2. *Microorganisms in the samples*

Sample ¹	Microorganism	Strain		
		SLV no. ²	Origin	Reference ³
A	<i>Bacillus cereus</i>	SLV-160	-	CCUG 45098
	<i>Providencia alcalifaciens</i>	SLV-045	-	CCUG 44809
	<i>Staphylococcus aureus</i>	SLV-350	-	CCUG 45099
B	<i>Enterococcus durans</i>	SLV-078	meat	CCUG 44816
	<i>Escherichia coli</i>	SLV-477	cheese	CCUG 43601
	<i>Serratia marcescens</i>	SLV-040	pond water	ATCC 13 880
	<i>Staphylococcus aureus</i>	SLV-280	egg	Egg, 1989
C	<i>Bacillus cereus</i>	SLV-518	couscous	CCUG 44741
	<i>Enterococcus faecium</i>	SLV-459	-	CCUG 35172
	<i>Staphylococcus xylosum</i>	SLV-283	cheese	-

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 for “Index of dispersion” between vials (I_2) and the test for reproducibility (T) do not simultaneously exceed 2.0 and 2.6, respectively. (For definitions of I_2 , and T, see references 6 and 7 respectively.)

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

Analysis and method	A ¹			B ¹			C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.99	2.03	1.32	5.00	2.19	1.32	5.34	1.52	1.26
Aerobic microorganisms, 20 °C NMKL method no. 86:2013	4.97	0.85	1.21	4.95	0.95	1.23	5.32	1.16	1.23
Contaminating microorganisms ISO method no. 13559:2002 IDF method no. 153:2002	5.04	0.74	1.18	5.02	4.94	1.19	5.28	0.82	1.20
Enterobacteriaceae NMKL method no. 144:2005	4.87	0.42	1.16	4.51	1.80	1.62	-	-	-
Coliform bacteria, 30 °C NMKL method no. 44:2004	4.88 ³	1.81 ³	1.36 ³	4.06	3.74	2.52	-	-	-
Coliform bacteria, 37 °C NMKL method no. 44:2004	4.85 ³	0.62 ³	1.20 ³	4.08	1.06	1.83	-	-	-
Thermotolerant coliform bacteria NMKL method no. 125:2005	-	-	-	4.11	2.66	1.52	-	-	-
<i>Escherichia coli</i> NMKL method no. 125:2005	-	-	-	4.11	2.66	1.52	-	-	-
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	4.28	0.06	1.12	-	-	-	4.31	2.42	1.94
Coagulase-positive staphylococci NMKL method no. 66:2009	3.91	0.83	1.22	4.52	0.12	1.13	5.19 ³	0.85 ³	1.23 ³
Enterococci NMKL method no. 68:2011	-	-	-	4.08	0.52	1.21	4.45	0.61	1.32
Gram-negative bacteria in pasteurised milk and cream. Detection of recontamination. NMKL method no. 192:2011	Pos.	-	-	Pos.	-	-	Neg.	-	-

– No target organism and therefore no value

¹ n = 5 vials analysed in duplicate

² n = 10 vials analysed in duplicate

³ Not target organism for the analysis

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58–64.
2. de Jong A.E.I., Eijhusen, G.P., Brouwer-Post, E.J.F., Grand, M., Johansson, T., Kärkkäinen, T., Marugg, J., in't Veld, P.H., Warmerdam, F.H.M., Wörner, G., Zicavo, A., Rombouts, F.M., Beumer, R.R. 2003. Comparison of media for enumeration of *Clostridium perfringens* from foods, *Journal of Microbiological Methods*, 54(3):359–366.
3. Byrne, B., Scannell, A.G.M., Lyng, J., Bolton, D.J. 2008. An evaluation of *Clostridium perfringens* media, *Food Control* 19(11):1091–1095
4. Anonymous, 2018. Protocol. Microbiology. Drinking water & Food, Swedish Food Agency.
5. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
6. 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.
7. 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.

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		A
9034	3	2	1	4.92	4.94	4.93	4.81	4.87	5	-	-	-	4.74	4.52	<1	-	-	-	-	-	-	-	-	-	<1	3.82	<1	-	-	-	-	-	-	-	-	-	-	-	-	9034	
9078	2	3	1	4.94	4.97	5.29	-	-	-	-	-	-	4.65	4.47	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078		
9217	2	1	3	5.06	5.06	5.39	-	-	-	-	-	-	4.93	4.81	<1	-	-	-	-	-	-	-	-	-	-	-	4.1	3.6	4.3	3.82	4.28	<1	<2	4.02	4.41	-	-	-	9217		
9269	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9269			
9429	3	1	2	-	-	-	-	-	-	-	-	-	4.78	4.54	<1	<1	4.04	<1	<1	4.2	<1	<1	4.18	<1	<1	4.18	<1	-	-	-	-	-	<1	4.04	4.54	-	-	-	9429		
9436	1	2	3	4.96	4.78	5.18	-	-	-	-	-	-	4.79	4.17	<1	<1	3.8	<1	<1	3.98	<1	<1	3.94	<1	<1	3.95	<1	4.11	<1	4.04	3.83	4.58	<1	<1	4.01	4.48	-	-	9436		
9453	1	3	2	4.91	4.75	5.15	-	-	-	4.75	4.86	4.88	4.86	4.49	<1	-	-	-	-	-	-	-	-	-	-	-	3.98	<1	4.1	3.85	4.6	<1	<1	4.11	4.55	-	-	-	9453		
9512	2	1	3	4.77	4.85	4.93	-	-	-	-	-	-	4.65	4.37	<1	-	-	-	-	-	-	-	-	-	-	-	<1	<1	1.95	-	-	-	-	-	-	-	-	-	9512		
9559	3	1	2	4.89	4.98	5.18	4.87	5.01	5.19	-	-	-	4.81	4.58	<1	-	-	-	-	-	-	-	-	-	<1	3.89	<1	4	<1	4.34	3.85	4.56	<1	-	-	-	Neg	Pos	Neg	9559	
9662	2	3	1	4.62	4.88	4.82	0	4.88	4.77	-	-	-	4.72	4.36	0	0	4.43	0	0	4.6	0	-	-	-	0	4.02	0	3.69	0	3.49	3.81	4.3	0	0	3.96	3.43	-	-	9662		
9747	2	3	1	4.66	5.79	5.18	-	-	-	-	-	-	4.64	5.22	<1	-	-	-	-	-	-	-	-	-	-	-	<1	<1	4.34	-	-	-	-	-	-	-	-	-	9747		
9890	2	3	1	4.89	4.79	5.4	4.96	4.88	5.3	-	-	-	4.67	4.64	4.23	-	-	-	-	-	-	-	-	-	0	4.18	0	4.04	0	4.49	3.74	4.43	0	-	-	-	-	-	9890		
9903	2	3	1	4.94	4.95	5.12	-	-	-	-	-	-	4.85	4.39	<1	-	-	-	-	-	-	-	-	-	<1	3.94	<1	3.97	<1	4.2	3.71	4.34	<1	<1	3.95	4.34	-	-	-	9903	
9950	3	2	1	4.98	5.01	5.19	-	-	-	-	-	-	4.56	4.57	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950		

N		162	161	161	24	24	24	13	14	14	145	143	144	40	39	40	88	86	87	45	45	46	118	115	117	112	110	112	96	96	98	60	61	61	10	10	10	N	
Min		4.08	4.36	4.61	0	4.49	4.41	4.73	4.57	4.21	0	0	0	0	3.24	0	0	0	0	0	3.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	Min
Max		5.88	6.00	6.10	5.18	5.15	5.53	5.03	5.18	5.41	5.91	5.69	4.49	4.93	4.80	0	4.86	5.11	4.45	4.80	4.66	0	4.57	4.98	3.91	5.03	4.74	5.27	4.91	5.42	6.07	4.38	4.99	4.99	-	-	-	Max	
Med		4.93	4.96	5.22	4.93	4.95	5.25	4.90	4.89	5.08	4.78	4.53	0	0	4.34	0	0	4.19	0	0	4.08	0	0	4.04	0	3.97	0	4.24	3.83	4.46	0	0	4.09	4.41	-	-	-	Med	
m		4.938	4.952	5.201	4.912	4.962	5.214	4.888	4.884	4.999	4.742	4.509	0	0	4.235	0	0	4.229	0	0	4.134	0	0	4.083	0	3.937	0	4.216	3.814	4.447	0	0	4.085	4.446	pos	pos	neg	m	
s		0.131	0.120	0.182	0.113	0.081	0.158	0.099	0.170	0.365	0.152	0.150	0	0	0.307	0	0	0.332	0	0	0.190	0	0	0.207	0	0.392	0	0.244	0.103	0.113	0	0	0.163	0.102	-	-	-	s	
u_(g)		0.010	0.010	0.014	0.024	0.017	0.033	0.027	0.045	0.098	0.013	0.013	0	0	0.049	0	0	0.036	0	0	0.029	0	0	0.020	0	0.039	0	0.024	0.011	0.012	0	0	0.021	0.014	-	-	-	u_(g)	
F+		0	0	0	0	0	0	0	0	0	0	0	4	7	0	0	16	0	2	1	0	0	2	0	1	0	5	0	0	0	0	5	1	0	0	0	0	0	F+
F-		0	0	0	1	0	0	0	0	0	5	1	0	0	0	0	0	1	0	0	0	0	0	0	0	12	0	3	1	1	0	0	1	3	1	0	0	0	F-
<		4	2	0	1	1	1	0	0	0	4	3	0	0	0	0	0	1	0	0	1	0	0	2	0	1	0	3	6	4	0	0	1	4	-	-	-	-	<
>		2	3	1	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2	5	2	0	0	1	2	-	-	-	-	>
< OK		4.60	4.61	4.61	4.69	4.83	4.77	4.73	4.57	4.21	4.20	4.00	0	0	3.24	0	0	3.04	0	0	3.86	0	0	3.61	0	2.78	0	3.41	3.52	4.14	0	0	3.63	4.29	-	-	-	< OK	
> OK		5.34	5.34	5.77	5.18	5.15	5.53	5.03	5.18	5.41	5.04	4.92	0	0	4.80	0	0	5.11	0	0	4.66	0	0	4.69	0	5.03	0	5.02	4.11	4.78	0	0	4.57	4.78	-	-	-	> OK	

N = number of analyses performed Max = highest reported result m = mean value F+ = false positive < = low outlier < OK = lowest accepted value u_(g) = 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.	Sample	Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorganisms			Enterobacteriaceae			Coliform bacteria 30 °C			Coliform bacteria 37 °C			Thermotolerant coliform bacteria			<i>Escherichia coli</i>			Presumptive <i>Bacillus cereus</i>			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					
9003	1 2 3	-1.281	0.236	-0.005																																		9003	
9007	2 3 1	0.468	0.319	-0.005																																			9007
9025	3 1 2	0.925	-0.265	-0.555																																			9025
9034	3 2 1	-0.140	-0.098	-1.489	-0.901	-1.145	-1.355																																9034
9078	2 3 1	0.012	0.152	0.489																																			9078
9217	2 1 3	0.925	0.904	1.039																																			9217
9269	3 1 2																																						9269
9429	3 1 2																																						9429
9436	1 2 3	0.164	-1.434	-0.115																																			9436
9453	1 3 2	-0.216	-1.684	-0.280																																			9453
9512	2 1 3	-1.275	-0.839	-1.464																																			9512
9559	3 1 2	-0.368	0.236	-0.115	-0.372	0.591	-0.151																																9559
9662	2 3 1	-2.422	-0.599	-2.093																																			9662
9747	2 3 1	-2.118	4.000	-0.115																																			9747
9890	2 3 1	-0.368	-1.350	1.094	0.422	-1.021	0.546																																9890
9903	2 3 1	0.012	-0.015	-0.445																																			9903
9950	3 2 1	0.316	0.486	-0.060																																			9950

The results are not evaluated

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