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Proficiency Testing **Microbiology – Food**

October 2017



Accred. no. 1457
Proficiency testing
ISO/IEC 17043

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 pasteurized dairy products

Abbreviations

Media

BA	Blood agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
BEA	Bile Esculin Agar
BcsA	<i>Bacillus cereus</i> selective Agar
BGLB	Brilliant Green Lactose Bile broth
BP	Baird-Parker agar
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
LTLSB	Lactose Tryptone Lauryl Sulphate Broth
MPCA	Milk Plate Count Agar
MYP	Mannitol egg Yolk Polymyxin agar
PCA	Plate Count Agar
RPF	Rabbit Plasma Fibrinogen
SFA	Sugar-Free Agar
TBX	Tryptone Bile X-glucuronide agar
TGE	Tryptone Glucose Extract agar
TSA	Tryptone Soya Agar
VRB	Violet Red Bile agar
VRBG	Violet Red Bile Glucose agar

Organisations

ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
SLV/NFA	Livsmedelsverket/National Food Agency, Sweden

Contents

General information on results evaluation.....	4
Results of the PT round October 2017	5
- General outcome	5
- Aerobic microorganisms, 20 °C and 30 °C	6
- Contaminating microorganisms in dairy products.....	9
- Enterobacteriaceae	11
- Coliform bacteria 30 °C and 37 °C.....	13
- Thermotolerant coliform bacteria and <i>Escherichia coli</i> ..	16
- Presumptive <i>Bacillus cereus</i>	19
- Coagulase-positive <i>Staphylococci</i>	21
- <i>Enterococci</i>	23
- Gram-negative bacteria in pasteurized dairy products	24
Outcome of the results of individual laboratory – assessment	26
- Box plot	27
Test material and quality control	33
- Test material	33
- Quality control of the mixtures	34
References	35
Annex 1: Results obtained by the participants	
Annex 2: z-scores of all participants	

General information on results evaluation

Statistical evaluation of the results

Highly deviating values that did not belong to a strictly normal distribution after \log_{10} transformation were identified as statistical outliers (Grubbs' test modified by Kelly (1)). In some cases, subjective adjustments were made to set limits, based on knowledge of the mixture's contents. Outliers and false results were not included in the calculations of means and standard deviations. Results reported as "> value" were excluded from the evaluation. Results reported as "< value" were interpreted as being zero (negative result). In cases where "Pos" and "Neg" were reported for quantitative analyses, they were treated as >1 and <1 respectively. All reported results are presented in Annex 1.

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



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

Uncertainty of measurement for the assigned values

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




Table and figure legends

Tables

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

Figures

Histograms of the analytical results for each mixture are presented. The mean value of the analysis results is indicated in each histogram.

	values within the interval of acceptance (Annex 1)
	outliers
	false negative results
*	values outside of the x-axis scale

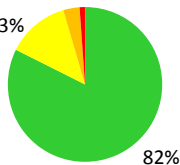
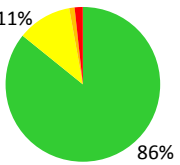
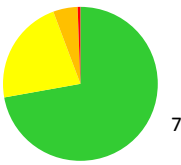
Results of the PT round October 2017

General outcome

Samples were sent to 189 laboratories, 49 in Sweden, 123 in other European countries, and 17 outside Europe. Of the 176 laboratories that reported results, 68 (39 %) provided at least one result that received an annotation. In the previous round with similar analyses (October 2016), the proportion was 50 %.

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

Table 1 Microorganisms in each mixture and % of deviating results (N: number of reported results, F%: false positive or false negative, X%: outliers).

		Mixture A				Mixture B				Mixture C			
% participants with													
Microorganisms		<i>Enterococcus hirae</i> <i>Klebsiella pneumoniae</i> <i>Micrococcus sp.</i>				<i>Escherichia coli</i> <i>Serratia marcescens</i> <i>Staphylococcus hyicus</i>				<i>Bacillus cereus</i> <i>Providencia alcalifaciens</i> <i>Staphylococcus aureus</i>			
Analysis		Target organism	N	F%	X%	Target organism	N	F%	X%	Target organism	N	F%	X%
Aerobic micro-organisms	30°C	All	167	1	6	All	167	0	3	All	168	0	2
	20°C	All	28	0	0	All	28	0	0	All	28	0	0
Contaminating microorganisms		All	17	0	6	All	17	0	6	All	17	0	6
Enterobacteriaceae		<i>K. pneumoniae</i>	142	2	4	<i>E. coli</i> <i>S. marcescens</i>	140	1	4	<i>P. alcalifaciens</i>	142	3	4
Coliform bacteria	30°C	<i>K. pneumoniae</i>	55	2	5	<i>E. coli</i>	54	2	6	(<i>P. alcalifaciens</i>)	55	16	0
	37°C	<i>K. pneumoniae</i>	93	1	0	<i>E. coli</i>	93	2	0	(<i>P. alcalifaciens</i>)	91	16	0
Thermotolerant coliform bacteria		<i>K. pneumoniae</i>	47	4	0	<i>E. coli</i>	47	0	4	-	47	4	0
<i>E. coli</i>		-	115	3	0	<i>E. coli</i>	113	2	6	-	115	0	0
Presumptive <i>B. cereus</i>		-	117	2	0	(<i>S. marcescens</i>) (<i>S. hyicus</i>)	116	2	0	<i>B. cereus</i>	117	3	3
Coagulase-positive <i>Staphylococci</i>		-	107	0	0	(<i>S. hyicus</i>)*	106	16	0	<i>S. aureus</i>	107	2	8
<i>Enterococci</i>		<i>E. hirae</i>	72	1	13	-	71	1	0	-	71	0	0
Gram-negative bacteria in dairy prod.		<i>K. pneumoniae</i>	11	0	0	<i>E. coli</i> <i>S. marcescens</i>	11	0	0	<i>P. alcalifaciens</i>	11	0	0

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

* the results are not evaluated

Aerobic microorganisms, 20 °C and 30 °C

Mixture A

The strains of *Micrococcus* sp., *Klebsiella pneumoniae* and *Enterococcus hirae* were target organisms for the analysis both at 20 °C and at 30 °C. The analyses were without problem for the majority of the laboratories, and the results were distributed around a distinct peak at both temperatures. No outliers or false negative results were reported for the analysis at 20 °C, but for the analysis at 30 °C four low and six high outliers were reported. One laboratory reported a false negative result at 30 °C.

Mixture B

The strains of *Escherichia coli*, *Serratia marcescens* and *Staphylococcus hyicus* were target organisms for the analysis both at 20 °C and at 30 °C. The analyses were without problem for the majority of the laboratories, and the results were distributed around a distinct peak at both temperatures. No outliers or false negative results were reported for the analysis at 20 °C. For the analysis at 30 °C two low and three high outliers were reported, but no false negative results.

Mixture C

The strains of *Providencia alcalifaciens*, *Staphylococcus aureus* and *Bacillus cereus* were target organisms for the analysis both at 20 °C and at 30 °C. The analyses were without problem for the majority of the laboratories, and the results were distributed around a distinct peak at both temperatures. No outliers or false negative results were reported for the analysis at 20 °C. For the analysis at 30 °C one high and three low outliers were reported, but no false negative results.

General remarks

The analyses were as a whole without problems for the laboratories. The choice of method or media had no effect on the outcome for either of the mixtures. Outliers and false negative results could not be attributed to the use of a specific method or media.

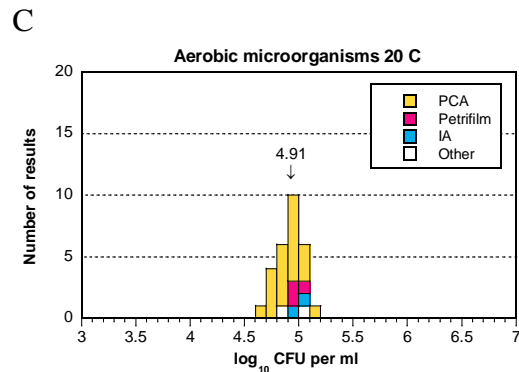
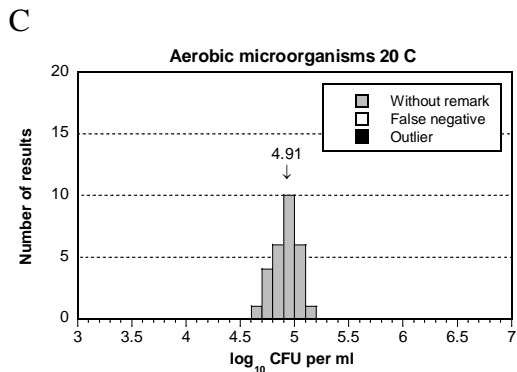
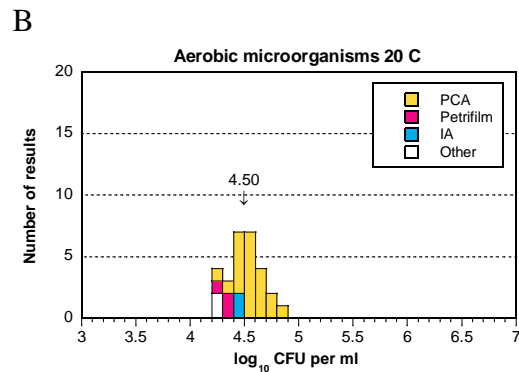
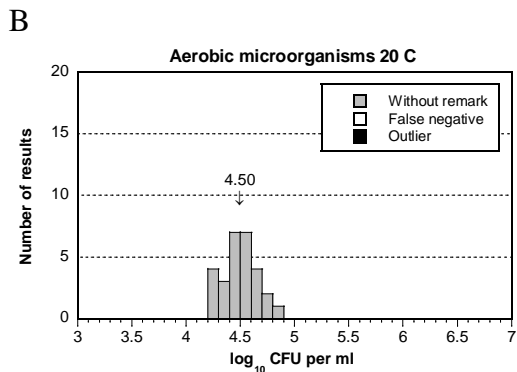
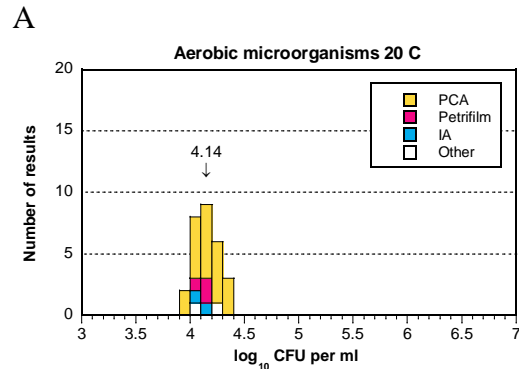
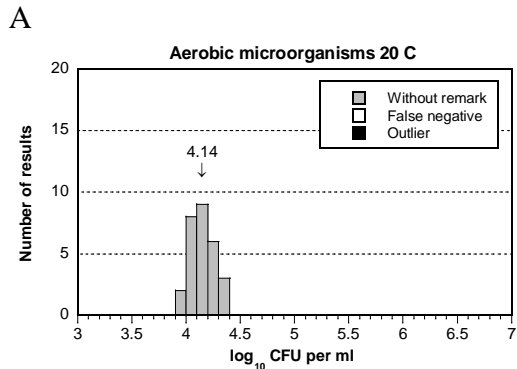
As in previous proficiency testing rounds, most laboratories followed either NMKL 86 or ISO 4833. Both methods recommend the use of Plate Count Agar (PCA), which was the most common media, followed by 3M™ Petrifilm™ Aerobic Count (Petrifilm AC), milk plate count agar (MPCA) and tryptone soya agar (TSA). Most laboratories followed the older versions NMKL 86:2006 and ISO 4833:2003, which have been replaced by NMKL 86:2013 and ISO 4833-1:2013 respectively. The incubation conditions are otherwise similar, and both the NMKL and ISO methods prescribe incubation for 72 h at 30 °C. For users of Petrifilm AC the incubation conditions depend on the method – for example AFNOR 3M 01/1-09/89 prescribes incubation for 72 h at 30 °C, whereas AOAC® 990.12 prescribes 48 h at 35 °C.

For the analysis at 20 °C two laboratories followed NMKL 184. This is a method for aerobic count and specific spoilage organisms in fish and fish products, and uses incubation on iron agar (IA).

For the analysis at 30 °C three laboratories used analyses based on the TEMPO® (bioMérieux® SA, Marcy l'Etoile, France) system, either TEMPO® AC or TEMPO® TVC. With these methods the sample is incubated in a card that contains wells with different volumes. A substrate in the card emits fluorescence when hydrolysed by the microorganisms. The determination of the number of microorganisms is based on MPN (Most Probable Number) and on the emitted fluorescence.

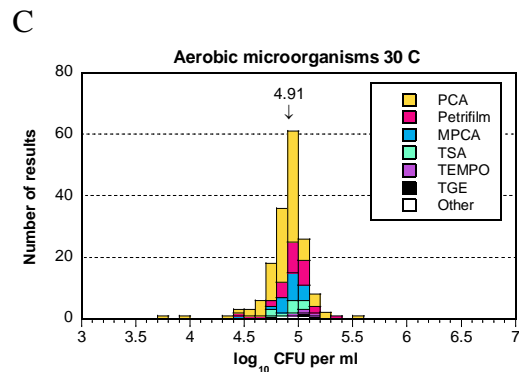
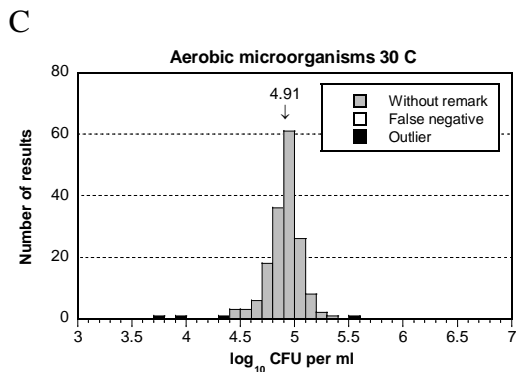
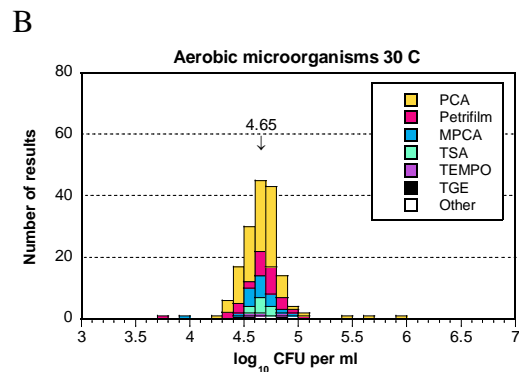
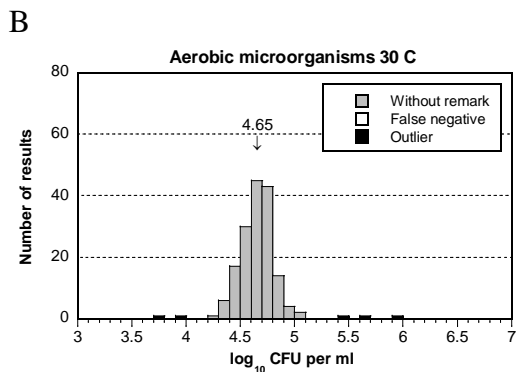
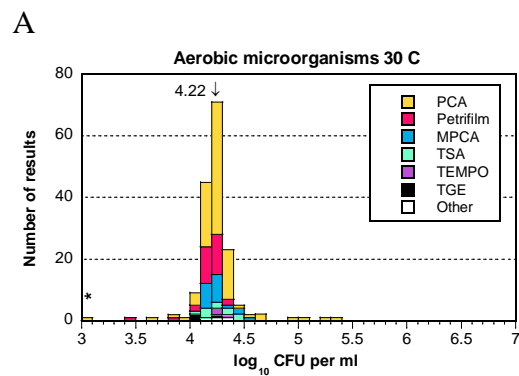
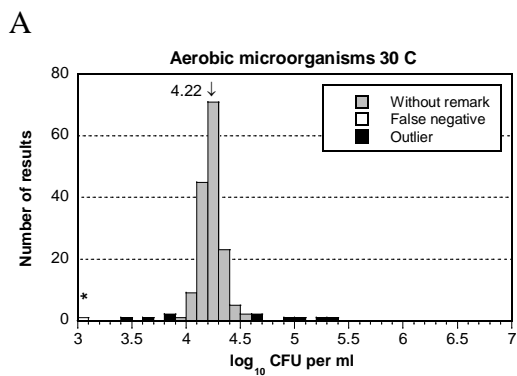
Results from analysis of aerobic microorganisms, 20 °C

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	28	28	4.14	0.11	0	0	0	28	4.50	0.15	0	0	0	28	4.91	0.12	0	0	0
PCA	21	21	4.15	0.12	0	0	0	21	4.55	0.14	0	0	0	21	4.89	0.13	0	0	0
Petrifilm AC	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0
IA	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0



Results from analysis of aerobic microorganisms, 30 °C

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	167	156	4.22	0.09	1	4	6	162	4.65	0.14	0	2	3	164	4.91	0.14	0	3	1
PCA	96	87	4.22	0.09	1	2	6	93	4.63	0.14	0	0	3	93	4.89	0.14	0	3	1
Petrifilm AC	31	29	4.19	0.07	0	2	0	30	4.67	0.17	0	1	0	31	4.93	0.17	0	0	0
MPCA	21	21	4.23	0.10	0	0	0	20	4.66	0.12	0	1	0	21	4.91	0.13	0	0	0
TSA	10	10	4.25	0.11	0	0	0	10	4.66	0.08	0	0	0	10	4.93	0.11	0	0	0
TEMPO	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0
TGE	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0
Other	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0



Contaminating microorganisms in dairy products

Mixture A

As in the analysis of aerobic microorganisms at 20 °C and 30 °C strains of *Micrococcus* sp., *Klebsiella pneumoniae* and *Enterococcus hirae* were target organisms. The results were distributed around a fairly distinct peak. The results from one laboratory was clearly lower than the rest, and should be considered an outlier. No false negative results were reported.

Mixture B

As in the analysis of aerobic microorganisms at 20 °C and 30 °C strains of *Escherichia coli*, *Serratia marcescens* and *Staphylococcus hyicus* were target organisms. The results were distributed around a fairly distinct peak. The results from one laboratory was clearly lower than the rest, and should be considered an outlier. No false negative results were reported.

Mixture C

As in the analysis of aerobic microorganisms at 20 °C and 30 °C strains of *Providencia alcalifaciens*, *Staphylococcus aureus* and *Bacillus cereus* were target organisms. The results were distributed around a distinct and narrow peak. The results from one laboratory was clearly lower than the rest, and should be considered an outlier. No false negative results were reported.

General remarks

Only 17 laboratories performed the analysis, which is therefore not evaluated statistically. Median values are therefore also shown instead of mean values in the table and figures below. The results are in general however in agreement with the concentrations determined in the analyses at the National Food Agency (Table 3).

The aim of the analysis is to identify potential contaminating bacteria in dairy products. Here nine of the 17 laboratories (53 %) stated that they followed the standard method ISO 13559:2002 / IDF 153:2002, while one laboratory used the older IDF 153:1991. The remaining laboratories either followed internal methods, or did not specify which method they used. All 17 laboratories however stated that they used sugar-free agar (SFA).

According to ISO 13559:2002 / IDF 153:2002, lactic acid bacteria are not classified as contaminating microorganisms in dairy products. Lactic acid bacteria are catalase negative, and can therefore be distinguished by a catalase test. Such as test is however not included in ISO 13559:2002 / IDF 153:2002, which only specifies the counting of colonies that are “characteristic contaminating microorganisms”. Five of the nine laboratories that followed ISO 13559/IDF 153 stated that they performed a catalase test. No other laboratories stated that they performed a confirmation. No obvious difference can however be found in the results between laboratories that performed a confirmation and those that did not.

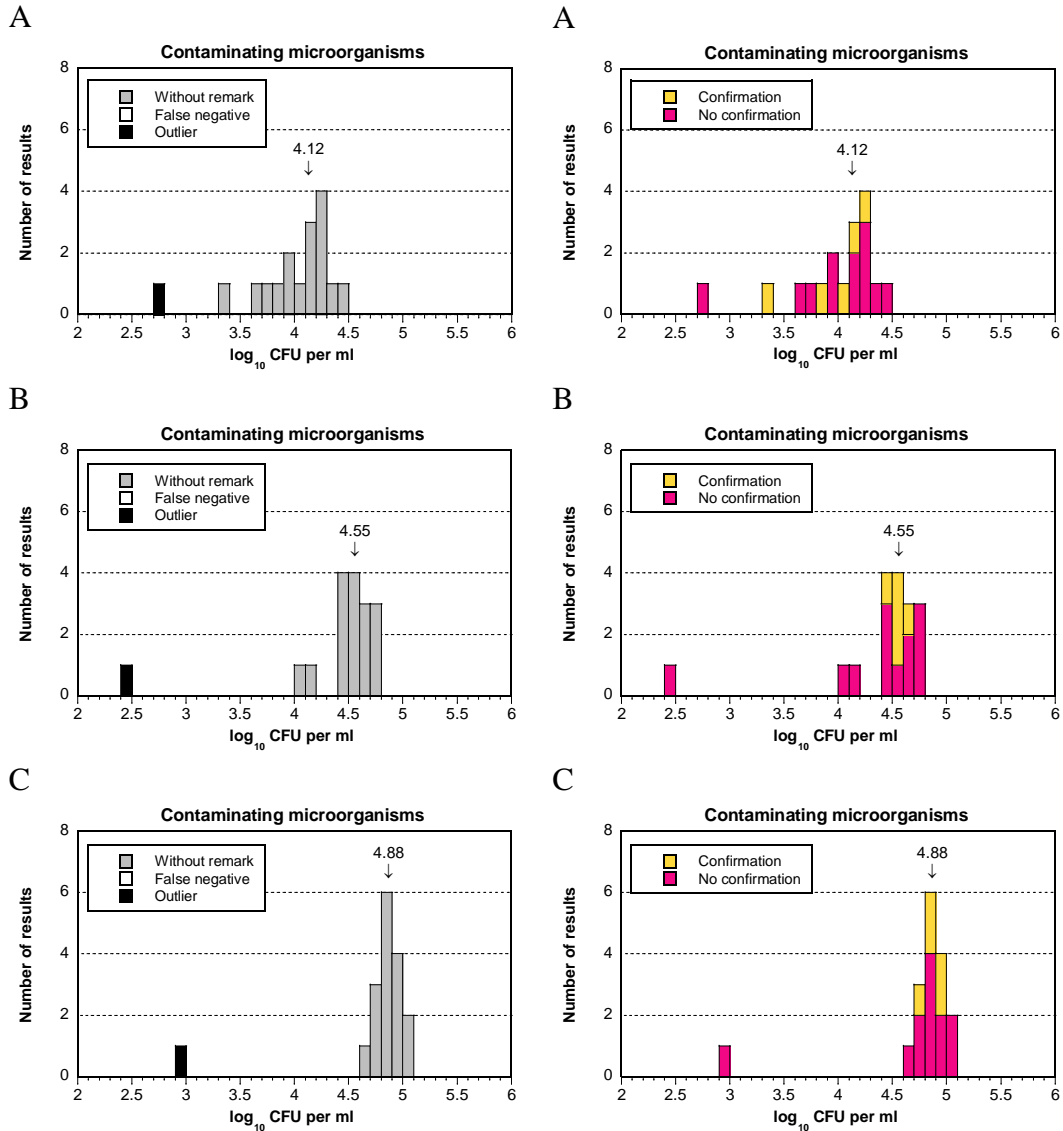
All three low outliers, one for each mixture, were reported by the same laboratory. According to ISO 13559:2002 / IDF 153:2002 small (pin-point) colonies shall be excluded in the enumeration of colonies. It is possible that laboratories have made different interpretations of “pin-point”, and that this could lead to lower results for laboratories that include fewer colonies.

Results from analysis of contaminating microorganisms in dairy products

Method	N	Mixture A					Mixture B					Mixture C							
		n	Med	s	F	<	>	n	Med	s	F	<	>	n	Med	s	F	<	>
All results	17	16	4.12	-	0	1	0	16	4.55	-	0	1	0	16	4.88	-	0	1	0
Confirmation	5	5	4.09	-	0	0	0	5	4.54	-	0	0	0	5	4.86	-	0	0	0
No confirmation*	12	11	4.18	-	0	1	0	11	4.55	-	0	1	0	11	4.89	-	0	1	0

Med: Median.

* "No confirmation" also includes two laboratories for which it is unclear if they performed a confirmation or not.



Enterobacteriaceae

Mixture A

The strain of *Klebsiella pneumoniae* was target organism for the analysis. The results were distributed around a distinct peak, with three low and two high outliers. Three laboratories reported false negative results.

Mixture B

The strains of *Escherichia coli* and *Serratia marcescens* were target organisms for the analysis. The results were distributed around a distinct peak, with four low and one high outlier. One laboratory reported a false negative result.

Mixture C

The strain of *Providencia alcalifaciens* was target organism for the analysis. The results were distributed around a distinct peak, with five low outliers. Four laboratories reported false negative results.

General remarks

The analyses were in general without problems for the 142 laboratories. The choice of method and medium had no effect on the outcome for either of the mixtures. Outliers and false negative results could not be attributed to the use of a specific method or medium.

Most laboratories reported following either NMKL 144:2005 (50 %), 3M Petrifilm (21 %) or ISO 21528-2:2004 (17 %). Two laboratories stated the use of ISO 21528-1:2004, which is a method based on MPN (Most Probable Number) for the analysis of Enterobacteriaceae. Both ISO 21528-1:2004 and ISO 21528-2:2004 have during 2017 been replaced by ISO 21528-1:2017 and ISO 21528-2:2017 respectively. The MPN method ISO 21528-1:2017 is recommended when the expected concentration of Enterobacteriaceae is lower than 100 cfu/gram.

Enterobacteriaceae are Gram-negative and oxidase negative bacteria, that ferment glucose with the production of acid by-products. On violet red bile glucose agar (VRBG) – which is used in both NMKL 144 and ISO 21528-2 – they form pink/red colonies, with or without a bile precipitation zone. The appearance is similar on 3M™ Petrifilm™ Enterobacteriaceae (Petrifilm EB), which also includes a colour indicator that assists in the detection of acid by-products, and a plastic film for detection of gas production.

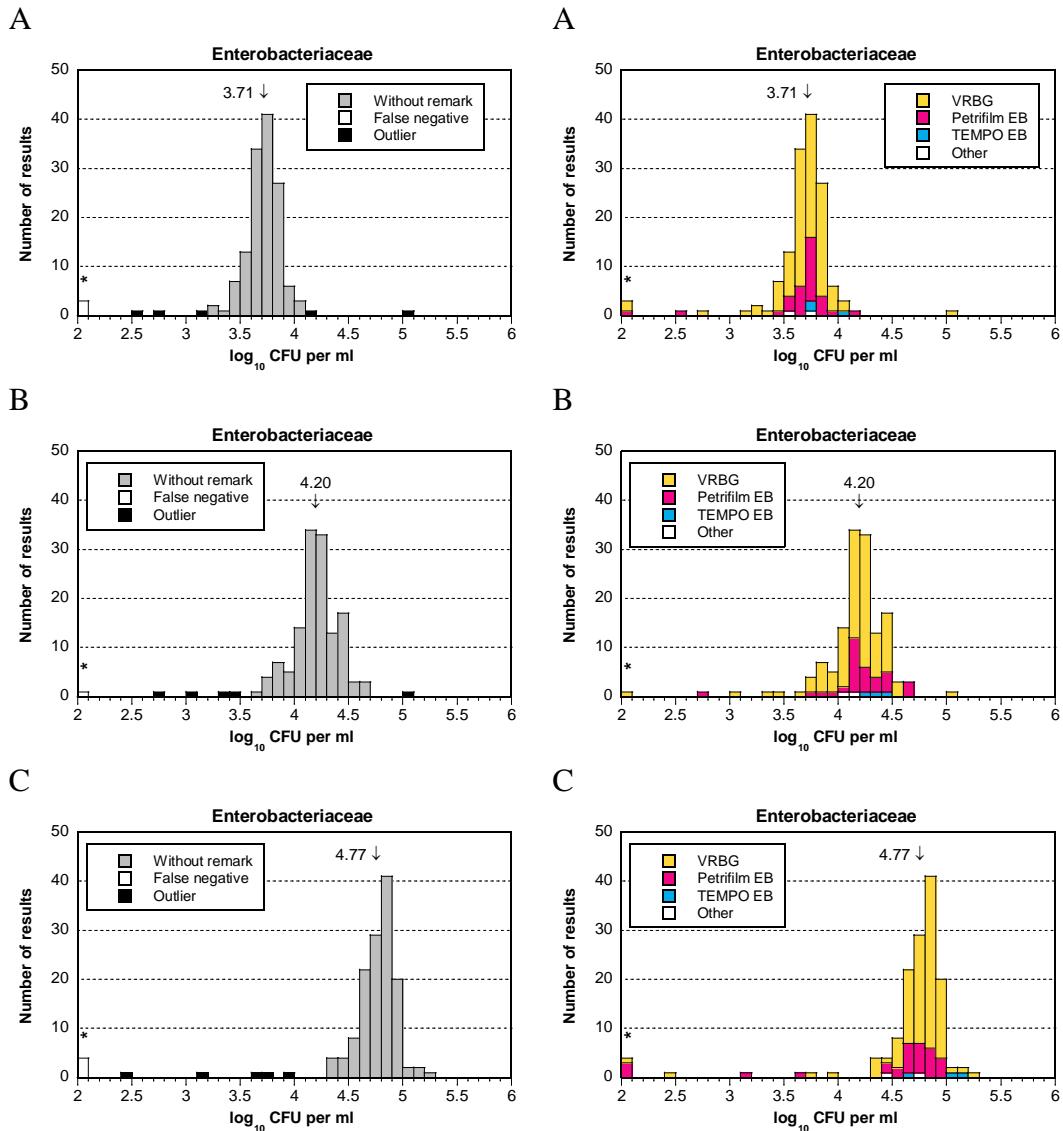
NMKL 144:2005 stipulates that presumptive colonies on VRBG shall be confirmed with an oxidase test. In contrast, ISO 21528-2:2004 states that presumptive colonies shall be confirmed with both an oxidase test and a test of glucose fermentation. In the revised ISO 21528-2:2017 the confirmation has been changed somewhat. In the new method, glucose agar has been replaced by glucose oxidation/fermentation (OF) medium. Colonies that are oxidase negative and that produce acid from glucose in the OF medium are confirmed as Enterobacteriaceae.

In total, 90 of the 142 laboratories (63 %) stated they performed some kind of confirmation, most commonly an oxidase test. Confirmation does not appear to have had an effect on the outcome. Rather, laboratories that did not perform a confirmation as a whole reported fewer false negative results and outliers compared to laboratories that performed a confirmation.

As in the analysis of aerobic microorganisms a small number of laboratories used fluorescence-based methods (TEMPO® Enterobacteriaceae). Though the results from this method appeared to be somewhat higher compared to VRBG and Petrifilm EB, users of TEMPO® reported neither outliers nor false positive results. The number of laboratories that used TEMPO® is at the same time too small to be thoroughly evaluated in this report.

Results from analysis of Enterobacteriaceae

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	142	134	3.71	0.14	3	3	2	134	4.20	0.19	1	4	1	133	4.77	0.16	4	5	0
VRBG	106	101	3.70	0.15	2	2	1	99	4.18	0.19	1	3	1	102	4.78	0.16	1	3	0
Petrifilm EB	31	28	3.71	0.10	1	1	1	30	4.23	0.21	0	1	0	26	4.75	0.13	3	2	0
TEMPO® EB	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0



Coliform bacteria, 30 °C and 37 °C

Mixture A

The strain of *Klebsiella pneumoniae* was target organism for the analysis at both 30 °C and 37 °C. The results for the analysis at 30 °C were distributed around a distinct peak. One low and two high outliers were reported. One laboratory reported a false negative result.

The results at 37 °C were also distributed around a distinct peak. At this temperature no outliers could be identified, but one laboratory reported a false negative result.

Mixture B

The strain of *Escherichia coli* was target organism for the analysis both at 30 °C and at 37 °C. The results for the analysis at 30 °C were distributed around a distinct but somewhat wide peak. Two low and one high outlier were reported. One laboratory reported a false negative result.

The results at 37 °C were also distributed around a somewhat wide peak, in which a smaller peak could possibly be seen for results with lower concentrations. No outliers could however be identified. In addition to *E. coli*, the mixture also contained a strain of *Staphylococcus hyicus*, and a strain of *Serratia marcescens*. *S. hyicus* is Gram-positive and should normally not grow on violet red bile agar (VRB) which was the most commonly used medium. *S. marcescens* is a weak fermenter of lactose and could therefore possibly form small colonies on VRB. The strain was present in the mixture at a concentration corresponding to that of the smaller peak (approximately log₁₀ 3.7 cfu/ml). At the National Food Agency two types of colonies were observed on VRB; large typical colonies of *E. coli*, and somewhat smaller colonies of likely *S. marcescens*. The smaller colonies were present in lower numbers, and were distinguished during the confirmation since they did not form gas in brilliant green lactose bile broth (BGLB). The results in the smaller peak were however reported both by laboratories that reported performing a confirmation, as well as laboratories that did not. Two laboratories reported false negative results for the analysis at 37 °C.

Mixture C

No target organism for the analysis was present in mixture C. The mixture did however contain a strain of *Providencia alcalifaciens*, which is false positive for the analysis. Nine of 55 laboratories reported false positive results at 30 °C and 15 of 91 laboratories at 37 °C. The majority of the false positive results were for concentrations around log₁₀ 4.7 cfu/ml, and corresponding to the concentration of *P. alcalifaciens* in the mixture. Not performing a confirmation appears to have contributed to the reporting of false positive results. As a whole, 73 % (30 °C) and 48 % (37 °C) of the laboratories reported performing some type of confirmation. Among those that reported false positive results, the corresponding number were in only 33 % (30 °C) and 27 % (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. A red/pink zone of precipitation is normally also formed due to the precipitation of bile salts when the pH decreases.

Further, the presence of bile salts and crystal violet in VRB inhibit the growth of Gram-positive microorganisms.

Most laboratories reported following either NMKL 44:2005 (28 %), ISO 4832:2006 (26 %), or the use of 3M™ Petrifilm™ (24 %). Both NMKL 44:2005 and ISO 4832:2006 stipulate incubation on VRB, but they differ somewhat in the confirmation step. NMKL 44:2004 states that all presumptive colonies on VRB shall be confirmed in BGLB, while ISO 4832:2006 states that only atypical colonies need to be confirmed. Further, if the sample is suspected to contain stressed coliform bacteria, NMKL 44:2004 recommends pre-incubation on tryptone soya agar (TSA). 3M™ Petrifilm™ Coliform Count (Petrifilm CC) is also based on VRB, and in addition includes a plastic film that facilitates detection of gas production.

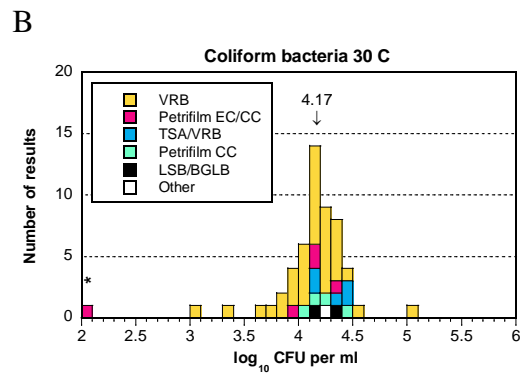
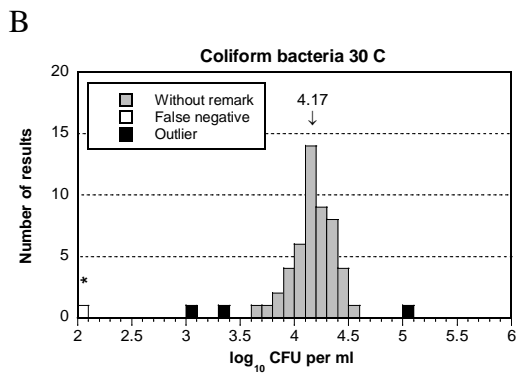
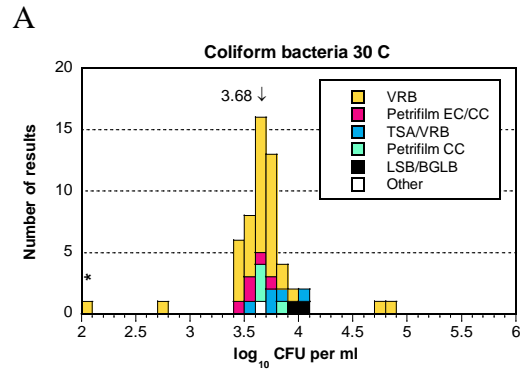
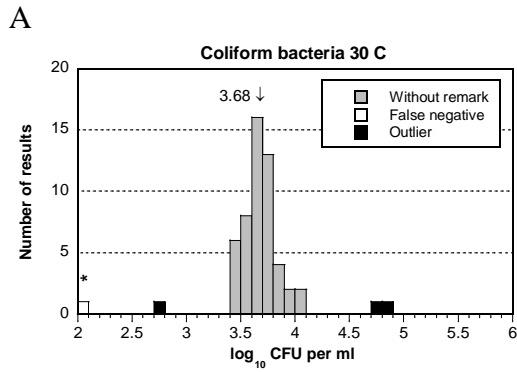
Lauryl sulphate broth (LSB) in combination with BGLB was used by laboratories that followed ISO 4831:2006 and NMKL 96 (different versions). ISO 4831:2006 is based on MPN (Most Probable Number) and is suitable to use when the expected concentration of coliform bacteria is lower than or equal to 100 cfu/g. NMKL 96 is also based on MPN, and 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.

For the analysis at 37 °C three laboratories used RAPID' *E. coli* 2 agar, which is a chromogenic medium that detects β-glucuronidase and β-galactosidase activity. On this medium coliform bacteria (Gal+/Gluc-) form blue/green colonies, while *E. coli* (Gal+/Gluc+) form pink/purple colonies. At the same temperature, two laboratories used Brilliance™ *E. coli*/coliform selective agar (Brilliance).

The mean values for TSA/VRB (30 °C and 37 °C) and LSB/BGLB (37 °C) differed somewhat from other media. These two media were however only used by five laboratories each, and it cannot be ruled out that the differences are due to random variation. Pre-incubation on TSA could however have contributed to the somewhat higher mean values for TSA/VRB.

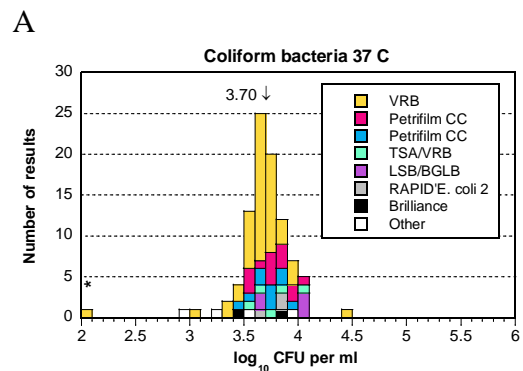
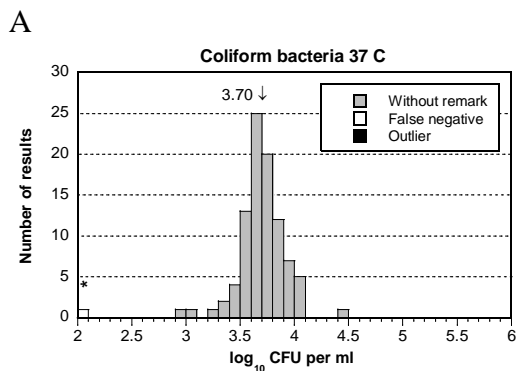
Results from analysis of coliform bacteria, 30 °C

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	55	51	3.68	0.15	1	1	2	50	4.17	0.18	1	2	1	46	-	-	9	-	-
VRB	38	34	3.65	0.13	1	1	2	34	4.14	0.19	0	2	1	34	-	-	4	-	-
Petrifilm EC/CC	5	5	3.58	0.12	0	0	0	4	4.14	0.13	1	0	0	3	-	-	2	-	-
TSA/VRB	5	5	3.78	0.17	0	0	0	5	4.30	0.16	0	0	0	5	-	-	0	-	-
Petrifilm CC	4	4	-	-	0	0	0	4	-	-	0	0	0	1	-	-	3	-	-
LSB/BGLB	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	-	-
Other	1	1	-	-	0	0	0	1	-	-	0	0	0	1	-	-	0	-	-

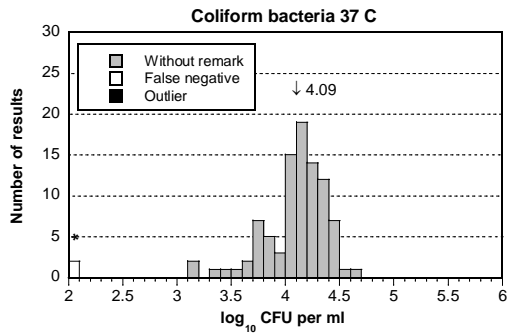


Results from analysis of coliform bacteria, 37 °C

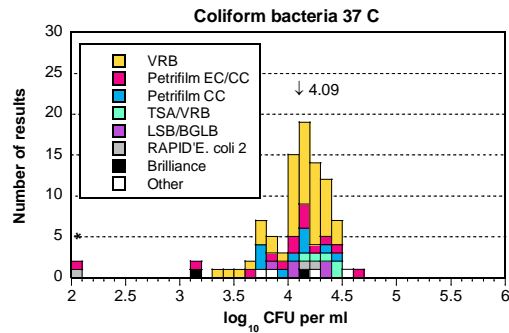
Medium	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	93	92	3.70	0.21	1	0	0	91	4.09	0.28	2	0	0	76	-	-	15	-	-
VRB	50	49	3.67	0.20	1	0	0	50	4.10	0.25	0	0	0	38	-	-	11	-	-
Petrifilm EC/CC	14	14	3.76	0.16	0	0	0	13	4.06	0.36	1	0	0	13	-	-	0	-	-
Petrifilm CC	10	10	3.73	0.14	0	0	0	10	4.05	0.25	0	0	0	7	-	-	3	-	-
TSA/VRB	5	5	3.75	0.19	0	0	0	5	4.32	0.17	0	0	0	5	-	-	0	-	-
LSB/BGLB	5	5	3.89	0.21	0	0	0	5	4.14	0.23	0	0	0	5	-	-	0	-	-
RAPID'E. coli 2	3	3	-	-	0	0	0	2	-	-	1	0	0	3	-	-	0	-	-
Brilliance	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	-	-
Other	4	4	-	-	0	0	0	4	-	-	0	0	0	3	-	-	1	-	-



B



B



Thermotolerant coliform bacteria

Mixture A

The strain of *Klebsiella pneumoniae* was target organism for the analysis of thermotolerant coliform bacteria. The results were distributed around a distinct peak. No outliers could be identified, but two laboratories reported false negative results.

No target organism for the analysis of *E. coli* was present in mixture A. Despite this, three laboratories reported false negative results.

Mixture B

The strain of *Escherichia coli* was target organism for the analysis of both thermotolerant coliform bacteria and *E. coli*. The results for thermotolerant coliform bacteria were distributed around a distinct peak, and two low outliers were reported. No false negative results were reported.

The results for the analysis of *E. coli* were distributed around a distinct but somewhat wide peak, with a tail of low outliers. In total six low and one high outlier were reported, as well as two false negative results. The outliers could not be attributed to the use of a specific method or medium. Outliers and false negative results were further reported both by laboratories that performed a confirmation test, as those that did not. The differences in incubation temperatures (mainly 37 °C or 44 °C) that were used by the laboratories did not appear to have an effect on the outcome.

Mixture C

No target organism was present in mixture C, neither for the analysis of thermotolerant coliform bacteria nor for *E. coli*. Despite this, two false positive results were reported for thermotolerant coliform bacteria. All laboratories that analysed *E. coli* however correctly reported negative results.

General remarks

As a whole, the analyses were without problem for the laboratories. No obvious differences based on the use of a specific method or medium could be seen in the analysis of thermotolerant coliform bacteria. Confirmation also did not appear to have had an effect on the outcome, neither for thermotolerant coliform bacteria nor for *E. coli*. As in the previous proficiency testing round (October 2016) several laboratories however reported unclear or ambiguous method information for the analysis of *E. coli*. Simultaneously, for this analysis several media were used by two or fewer laboratories,

and hence the group of “Other” is rather large. This also means that comparisons between different methods and media are more general for this analysis.

The most used method in both analyses was NMKL 125:2005. It describes the analysis of both thermotolerant coliform bacteria and of *E. coli*. The method defines thermotolerant coliform bacteria as those that form typical dark red colonies surrounded by a zone of precipitation on VRB after incubation for 24 h at 44 °C. Presumptive colonies are confirmed by inoculation either in *E. coli*-broth (EC) or in lactose tryptone lauryl sulphate broth (LTL SB). In both of these media, thermotolerant coliform bacteria produce gas as a consequence of lactose fermentation. *E. coli* are further defined as those thermotolerant coliform bacteria that also produce indole either in LTL SB or in tryptone broth.

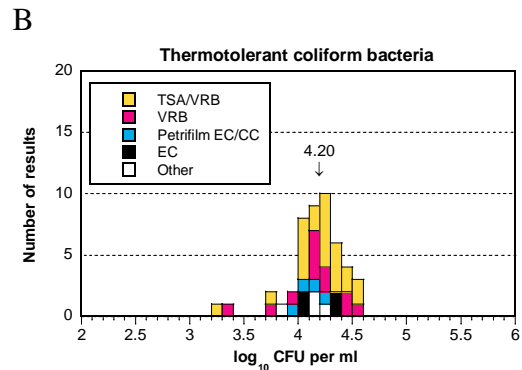
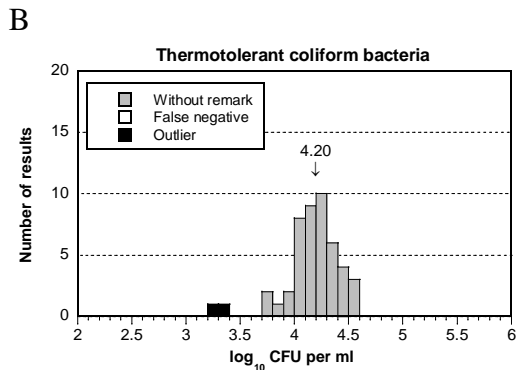
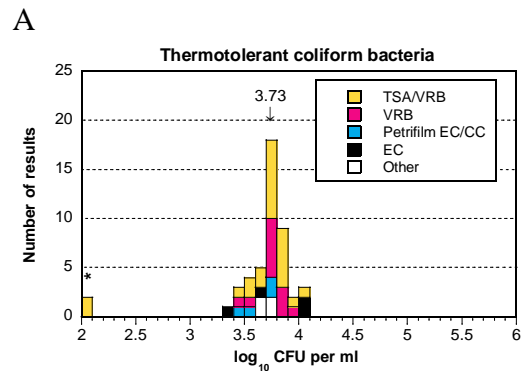
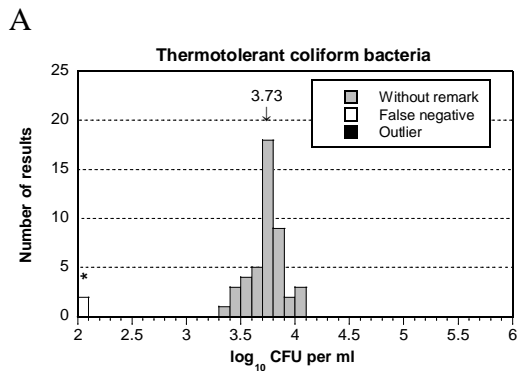
NMKL 125:2005 was the most used method also for the analysis of *E. coli*. It was followed by 3M™ Petrifilm™ and ISO 16649-2:2001. In ISO 16649-2:2001 *E. coli* are defined as those bacteria that form typical blue colonies on tryptone bile X-glucuronide agar (TBX) after 18-24 h at 44 °C. The colonies are stained blue due to the reaction between *E. coli* β -glucuronidase and an indicator in the medium. No further confirmation of β -glucuronidase positive colonies is required according to ISO 16649-2:2001. 3M™ Petrifilm™ EC/CC and 3M™ Petrifilm™ SEC are also based on media that detect *E. coli* β -glucuronidase activity. Further, the plastic film in Petrifilm EC/CC and Petrifilm SEC facilitates the detection of gas production due to lactose fermentation. Here, it should also be mentioned that NMKL 125 is currently being revised, and the new version will likely be more similar to ISO 16649-2.

In the analysis of *E. coli*, several of the reported methods were used only by a small number of laboratories. Four laboratories used methods based on the detection of fluorescence (TEMPO® *E. coli*). Three laboratories followed NMKL 96 (different versions) which is an MPN-based method adapted for the analysis of coliform bacteria, thermotolerant coliform bacteria and *E. coli* in fish and seafood. Three laboratories followed ISO 7251:2005, which is another MPN-based method for the detection of *E. coli*.

As in previous proficiency testing rounds, in the analysis of *E. coli* the results were somewhat lower for TBX, and somewhat higher for TSA/VRB, compared to other media. A possible explanation could be if the samples were been pre-incubated or not. If the presence of stressed microorganisms in the sample is suspected, ISO 16649-2:2001 stipulates that a pre-incubation should be carried out at 37 °C for 4 h, before the final incubation at 44 °C for 18-24 h. In comparison, a similar pre-incubation is routinely carried out in NMKL 125:2005 (1-2 h on TSA at 20-25 °C) prior to the final incubation on VRB. However the differences were small and, as mentioned above, the method data was unclear or ambiguous for several laboratories. The results were at the same time fairly spread out, especially for Petrifilm EC/CC and TBX.

Results from analysis of thermotolerant coliform bacteria

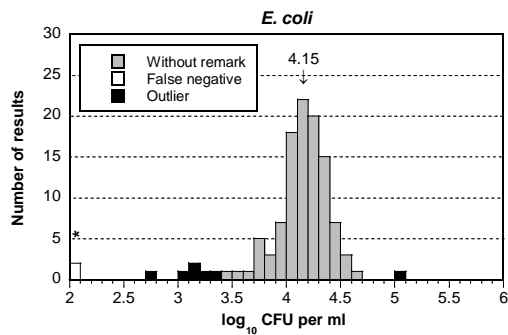
Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	47	45	3.73	0.15	2	0	0	45	4.20	0.19	0	2	0	45	-	-	2	-	-
TSA/VRB	23	21	3.75	0.13	2	0	0	22	4.22	0.17	0	1	0	22	-	-	1	-	-
VRB	12	12	3.74	0.14	0	0	0	11	4.21	0.23	0	1	0	12	-	-	0	-	-
Petrifilm EC/CC	4	4	-	-	0	0	0	4	-	-	0	0	0	3	-	-	1	-	-
EC	4	4	-	-	0	0	0	4	-	-	0	0	0	4	-	-	0	-	-
Other	4	4	-	-	0	0	0	4	-	-	0	0	0	4	-	-	0	-	-



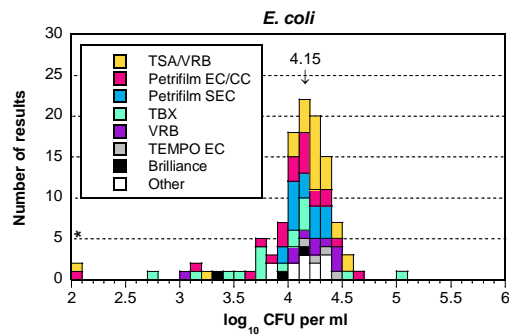
Results from analysis of Escherichia coli

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	115	112	-	-	3	-	-	104	4.15	0.21	2	6	1	115	-	-	0	-	-
TSA/VRB	26	26	-	-	0	-	-	24	4.24	0.14	1	1	0	26	-	-	0	-	-
Petrifilm EC/CC	22	21	-	-	1	-	-	20	4.10	0.23	1	1	0	22	-	-	0	-	-
Petrifilm SEC	19	19	-	-	0	-	-	19	4.15	0.13	0	0	0	19	-	-	0	-	-
TBX	18	18	-	-	0	-	-	14	3.93	0.29	0	2	1	18	-	-	0	-	-
VRB	10	8	-	-	2	-	-	9	4.29	0.17	0	1	0	10	-	-	0	-	-
TEMPO EC	4	4	-	-	0	-	-	4	-	-	0	0	0	4	-	-	0	-	-
Brilliance	3	3	-	-	0	-	-	2	-	-	0	1	0	3	-	-	0	-	-
Other	13	13	-	-	0	-	-	12	4.15	0.18	0	0	0	13	-	-	0	-	-

B



B



Presumptive *Bacillus cereus*

Mixture A

No target organism for the analysis was present in mixture A. Two laboratories reported false positive results.

Mixture B

No target organism for the analysis was present in mixture A. Two laboratories reported false positive results. Likely, they have detected either *Serratia marcescens* (approximately log₁₀ 3.7 cfu/ml in the mixture) or *Staphylococcus hyicus* (approximately log₁₀ 4.3 cfu/ml in the mixture).

Mixture C

The strain of *Bacillus cereus* was target organism for the analysis. The results for the 117 laboratories that performed the analysis were distributed around as distinct peak. Four low outliers were reported, as well as four false negative results.

General remarks

As a whole, the analyses were without major problems for the laboratories. The exceptions were the low outliers and the false negative results for mixture C. No obvious explanation could be found for the false negative results. It can however be noted that all four of the low outliers were reported by laboratories that did not perform a confirmation. At the same time, performing or not performing a confirmation does not appear to have had an effect on the outcome for mixtures A and B.

Most laboratories followed either NMKL 67:2010 (56 %) or ISO 7932:2004 (22 %). One laboratory used the older NMKL 67:2003. The remaining methods were either internal methods, method that were not specified, or methods that were only used by a few laboratories.

The most commonly used method NMKL 67:2010 is based on incubation on blood agar (BA). On BA, *B. cereus* forms large irregular grey colonies, that are surrounded by a large zone of haemolysis. Suspected colonies are confirmed either on *Bacillus cereus*-selective agar (BcsA) or on Cereus-Ident agar (a chromogenic medium). On BcsA presumptive *B. cereus* form bluish colonies that are surrounded by a 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 present in Cereus-

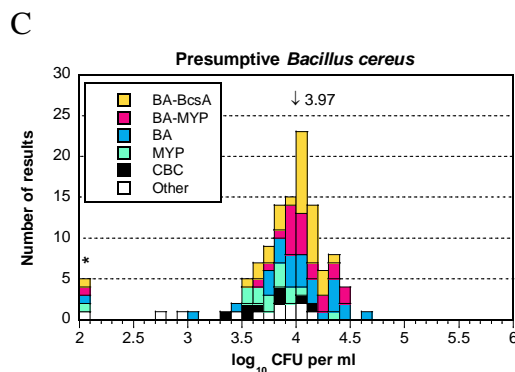
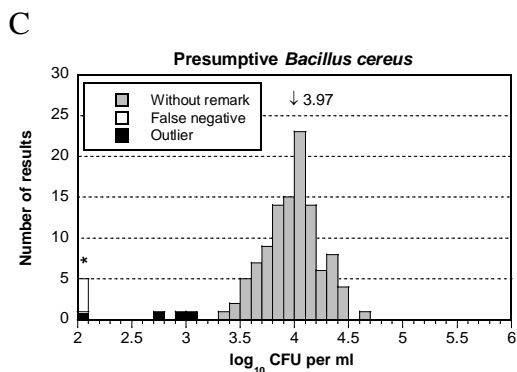
Ident agar. In comparison, ISO 7932:2004 prescribes plating onto mannitol egg yolk polymyxin agar (MYP). On MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity. The colonies are confirmed if they display haemolysis on BA.

An in previous proficiency testing rounds, the reported method data for the analysis of *B. cereus* was ambiguous for several laboratories. Several laboratories reported that the same medium was used in both steps in the analysis. Other laboratories reported combinations of method and media that were incompatible. As a general rule, the tables and figures below are based on the methods/media stated by the laboratories, regardless if these are compatible or not. In some cases it has however been assumed that the laboratory used the medium that is prescribed by the method. Laboratories that have only stated “chromogenic medium” are included in the group “Other”.

Despite the uncertainties in the reporting of method data, the mean values for the different media are very similar, with two exceptions. Oxoid Brilliance™ *Bacillus cereus* agar (CBC) was used by a group of eight laboratories. CBC is a chromogenic medium, and cleavage of X-Gluc present in CBC by *B. cereus* β-glucuronidase results in white colonies with a blue/green centre. The mean value for CBC was slightly lower compared to other media, but at the same time users of this medium reported neither outliers nor false negative results. The mean value for MYP was also slightly lower compared to other media. The deviations for both CBC and MYP are however in the same range as the variations commonly found among media in the analysis of *B. cereus* (e.g. April 2017 and April 2016).

Results from analysis of presumptive *Bacillus cereus*

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	117	115	-	-	2	-	-	114	-	-	2	-	-	109	3.97	0.25	4	4	0
BA-BcsA	31	31	-	-	0	-	-	30	-	-	0	-	-	30	4.01	0.19	1	0	0
BA-MYP	23	23	-	-	0	-	-	23	-	-	0	-	-	22	4.06	0.22	1	0	0
BA	28	27	-	-	1	-	-	26	-	-	2	-	-	26	4.06	0.28	1	1	0
MYP	14	14	-	-	0	-	-	14	-	-	0	-	-	13	3.81	0.21	1	0	0
CBC	8	8	-	-	0	-	-	8	-	-	0	-	-	8	3.74	0.26	0	0	0
Other	13	12	-	-	1	-	-	13	-	-	0	-	-	10	3.86	0.21	0	3	0



Coagulase-positive *Staphylococci*

Mixture A

No target organism for the analysis was present in mixture A. All of the 107 laboratories that performed the analysis correctly reported negative results.

Mixture B

No target organism for the analysis was present in mixture B. Despite this, 17 laboratories reported positive results. The majority of these reported concentrations corresponding to that of *Staphylococcus hyicus*, which was present in the mixture at approximately \log_{10} 4.3 cfu/ml. At the National Food Agency, the strain formed grey/white colonies without a zone of precipitation on Baird-Parker agar with rabbit plasma fibrinogen (BP + RPF). In subsequent confirmation the strain displays no, or only weak, coagulase activity.

Mixture C

The strain of *Staphylococcus aureus* was target organism for the analysis. The results were distributed around a distinct peak. Six low and three high outliers were reported, as well as two false negative results.

General remarks

As in previous proficiency testing rounds most laboratories (44 %) reported following NMKL 66:2009. The remaining laboratories either followed ISO 6888-1:1999 (17 %), used 3M™ Petrifilm™ Staph Express (15 %) or followed ISO 6888-2:1999 (9 %). Three laboratories used a fluorescence-based detection with TEMPO® STA.

NMKL 66:2009 prescribes incubation on BP and/or BP + RPF. The method also allows incubation on blood agar (BA) as a complement to these media. On BP, *S. aureus* forms characteristic convex shiny colonies, that have a grey/black colour due to reduction of tellurite in the medium. They are normally 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 NMKL 66 the colonies are confirmed by a positive result in a coagulase test. When BP + RPF is used, the coagulase activity is tested directly in the medium, and no further confirmation is required according to the method. Similar to NMKL 66, ISO 6888-1 stipulates plating onto BP and confirmation with a coagulase test, whereas ISO 6888-2 is based on BP + RPF. 3M™ Petrifilm™ Staph Express (Petrifilm Staph) uses a modified Baird-Parker medium, and also contains a chromogenic indicator that stains colonies of *S. aureus* red/purple.

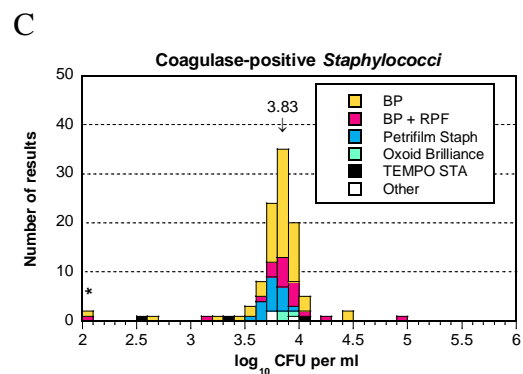
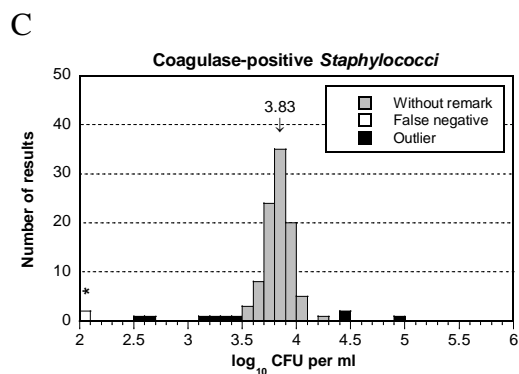
As a whole, the analyses were without problem for the laboratories. The exception was the large number of positive results reported for mixture B. These are likely a consequence of the characteristics of the particular strain that was used. *S. hyicus* is normally included among coagulase-positive *Staphylococci*, but the strain present in mixture B is in tests at the National Food Agency coagulase-negative, or only weakly coagulase-positive. Traditionally, coagulase-positive *Staphylococci* are confirmed 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. The majority of the positive

results in mixture B were reported by laboratories that used Petrifilm Staph, whereof most reported performing a confirmation with 3M™ Petrifilm™ Staph Express Disk (Petrifilm Disk). This is based on detection of extracellular DNase, which is produced by the majority of coagulase-positive *S. aureus*, but also by *S. intermedius* and *S. hyicus*. Toluidin blue O in the disks visualises DNase activity as a pink zone around the colonies. The current strain of *S. hyicus* is at the National Food Agency only characterised with media and confirmation methods based on rabbit plasma, and it cannot be ruled out that there is a variation in how it performs on other types of media and with other methods for confirmation. In conclusion, both negative results based on confirmation with a coagulase test, and positive results based on confirmation with Petrifilm Disk should therefore be considered correct. Due to the characteristics of the strain the results for mixture B are not evaluated further, and no z values are calculated for the analysis. The results for mixture B are also not included in the tables below the box plots.

Results from analysis of coagulase-positive *Staphylococci*

Medium	N	Mixture A					Mixture B*					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	107	107	-	-	0	-	-	89	-	-	17	-	-	96	3.83	0.11	2	6	3
BP	60	60	-	-	0	-	-	55	-	-	4	-	-	54	3.83	0.10	1	3	2
BP + RPF	20	20	-	-	0	-	-	20	-	-	0	-	-	17	3.87	0.13	1	1	1
Petrifilm Staph	18	18	-	-	0	-	-	6	-	-	12	-	-	18	3.74	0.09	0	0	0
Oxoid Brilliance Staph 24	3	3	-	-	0	-	-	3	-	-	0	-	-	3	-	-	0	0	0
TEMPO® STA	3	3	-	-	0	-	-	3	-	-	0	-	-	1	-	-	0	2	0
Other	3	3	-	-	0	-	-	2	-	-	1	-	-	3	-	-	0	0	0

* The results for mixture B are not evaluated



Enterococci

Mixture A

The strain of *Enterococcus hirae* was target organism for the analysis. The results for the 72 laboratories that performed the analysis were distributed around a distinct peak. Three low and six high outliers were reported, as well as one false negative result.

Mixture B

No target organism for the analysis was present in mixture B. One laboratory reported a false negative result.

Mixture C

No target organism for the analysis was present in mixture C. All laboratories that performed the analysis correctly reported negative results.

General remarks

As a whole, the analysis was without major problems for the laboratories, with the exception of a fairly high number of outliers for mixture A. The choice of method or media had no effect on the outcome for either of the mixtures. Confirmation also did not appear to have an effect on the outcome.

NMKL 68:2011 was by far the most common method and was used by the majority of the laboratories (71 %). In addition to this, IDF 149A:1997 was used by four laboratories (6 %) and ISO 7899-2:2000 by three laboratories (4 %). The remaining laboratories either used internal methods or did not specify which method they used.

Enterococci are in NMKL 68:2011 defined as Gram-positive, coagulase negative and oval cocci, that hydrolyse esculin at 44 °C. The method prescribes incubation on Slanetz & Bartley *Enterococcus*-agar (ENT). On ENT, enterococci reduce the colourless substrate 2,3,5-trifenyltetrazolium chloride to red formazan and form slightly raised colonies with a pink/red/maroon colour. They can sometimes also have a colourless edge. When stressed enterococci are suspected (e.g. in frozen foods) a pre-incubation on TSA for two hours is recommended, followed by overlay with ENT. Distinctly dark red colonies with a typical morphology are counted as enterococci without further confirmation. Colonies with a faint pink/red colour are confirmed by plating onto bile esculin agar (BEA). On BEA the substrate esculin is hydrolysed by β -glucosidase present in enterococci, which results in the formation of esculetin and glucose. Esculetin together with iron ions present in the medium then form a black precipitate. Incubation on BEA is at vid 44 °C and colonies that cause a blackening of the medium after 2-24 hours are counted as enterococci. Four laboratories analysed according to the drinking water method ISO 7899-2:2000, which is similar to NMKL 68:2001. The method is based on membrane filtering followed by incubation on ENT. Confirmation is, as in the NMKL method, by plating on BEA (possibly with the addition of azide), but incubation is only for 2 hours.

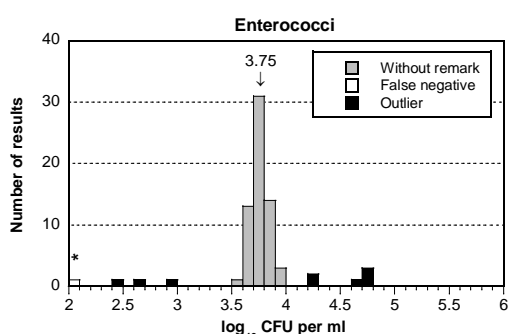
In addition to ENT, kanamycin esculin azide agar (KEAA) and COMPASS® *Enterococcus* agar were used by three laboratories each. KEAA was used by laboratories that followed IDF 149A:1997, and with this medium the hydrolysis of esculin is tested directly. Two of the three laboratories that used KEAA stated they also performed a subsequent confirmation, but did not specify this further. IDF 149:A:1997 has according to ISO also been replaced by ISO 27205:2010/IDF 149:2010. As with

BEA, COMPASS® *Enterococcus* Agar detects β-glucosidase activity, but the latter medium instead uses X-Gluc as substrate, resulting in the formation of blue colonies.

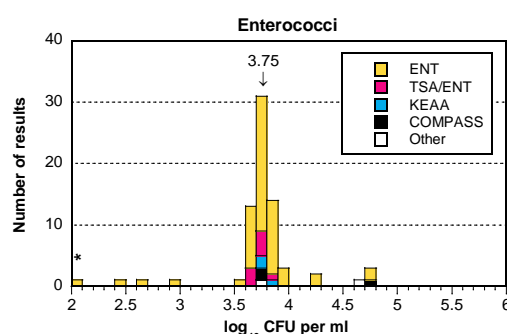
Results from analysis of Enterococci.

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	72	62	3.75	0.08	1	3	6	70	-	-	1	-	-	71	-	-	0	-	-
ENT	56	48	3.75	0.09	1	3	4	54	-	-	1	-	-	55	-	-	0	-	-
TSA/ENT	8	8	3.72	0.06	0	0	0	8	-	-	0	-	-	8	-	-	0	-	-
KEAA	3	3	-	-	0	0	0	3	-	-	0	-	-	3	-	-	0	-	-
COMPASS	3	2	-	-	0	0	1	3	-	-	0	-	-	3	-	-	0	-	-
Other	2	1	-	-	0	0	1	2	-	-	0	-	-	2	-	-	0	-	-

A



A



Gram-negative bacteria in dairy products

Mixture A

The strain of *Klebsiella pneumoniae* was target organism for the analysis. All eleven laboratories correctly reported positive results.

Mixture B

The strains of *Escherichia coli* and *Serratia marcescens* were target organism for the analysis. All eleven laboratories correctly reported positive results.

Mixture C

The strain of *Providencia alcalifaciens* was target organism for the analysis. All eleven laboratories correctly reported positive results.

General remarks

The analysis was without problems for the laboratories. All eleven laboratories reported using violet red bile glucose agar (VRBG) as medium and nine laboratories reported following NMKL 192:2011.

The method in NMKL 192:2011 is used to detect recontamination of Gram-negative bacteria in pasteurised milk and cream. Gram-negative 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 which may limit the shelf-life of the product. With NMKL

192:2011 the unopened package of milk/cream is incubated at 25 °C for 24 h, or at room temperature for 28 h. Subsequently 10 and 100 µl, respectively, are plated onto VRBG at 30 °C for 24 h. The presence of five or more colonies is considered a positive result. When needed, confirmation can be done with potassium hydroxide (KOH). Colonies are then transferred with a loop onto a glass slide with KOH, and colonies that after 5-10 seconds form a viscous string are considered as Gram-negative.

Results from analysis of Gram-negative bacteria in dairy products

Method	N	Mixture A		Mixture B		Mixture C	
		n	F	n	F	n	F
All results	11	11	0	11	0	11	0
NMKL 192:2011	9	9	0	9	0	9	0
Other	2	2	0	2	0	2	0

Outcome of the results of individual laboratory - assessment

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 only 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

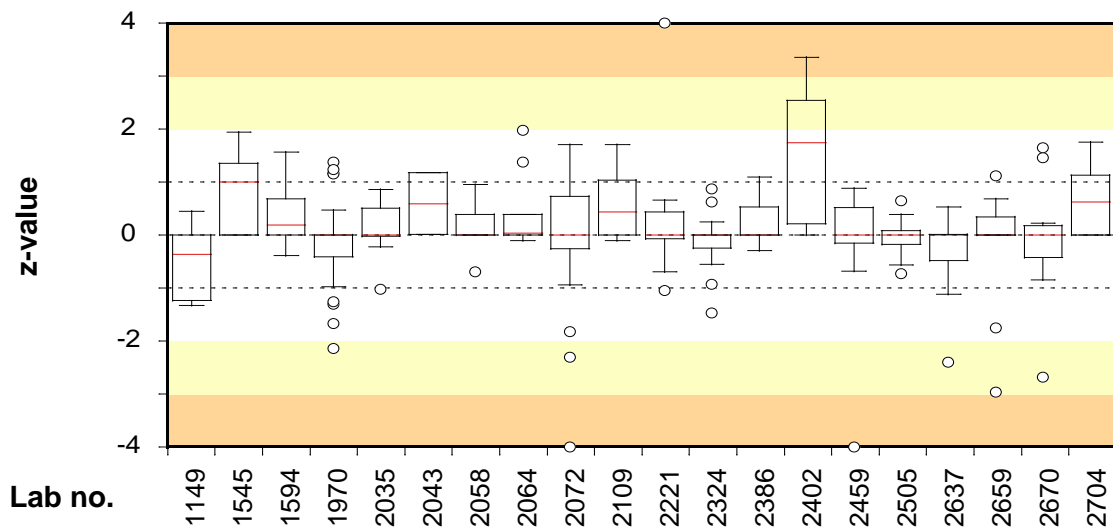
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

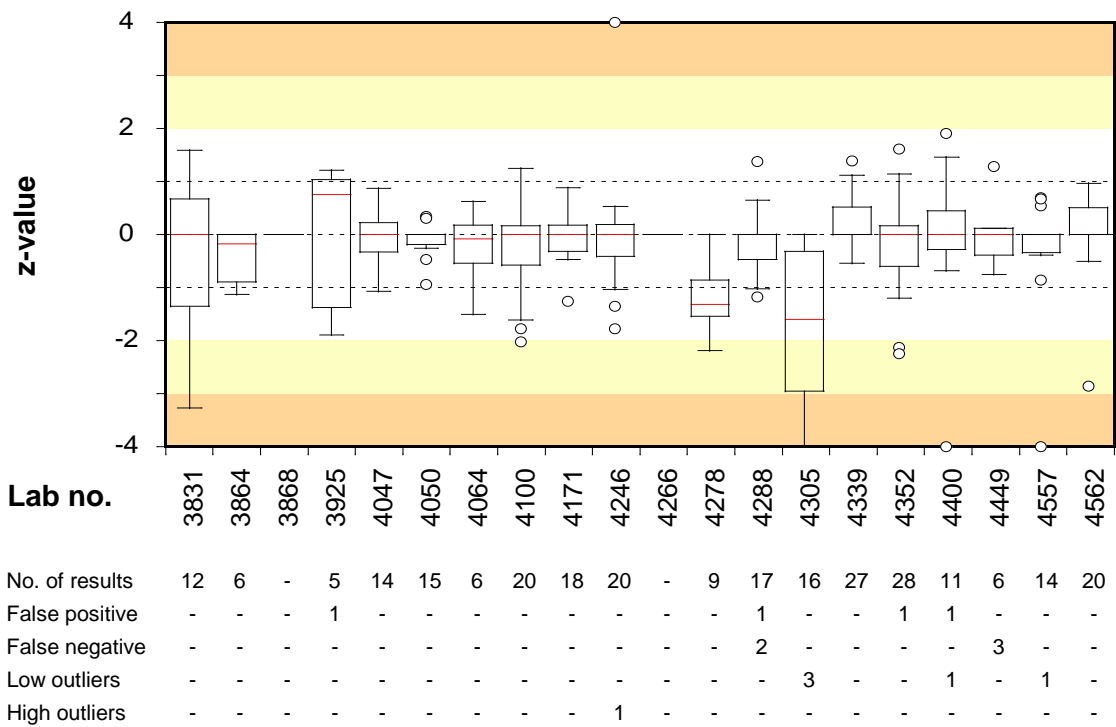
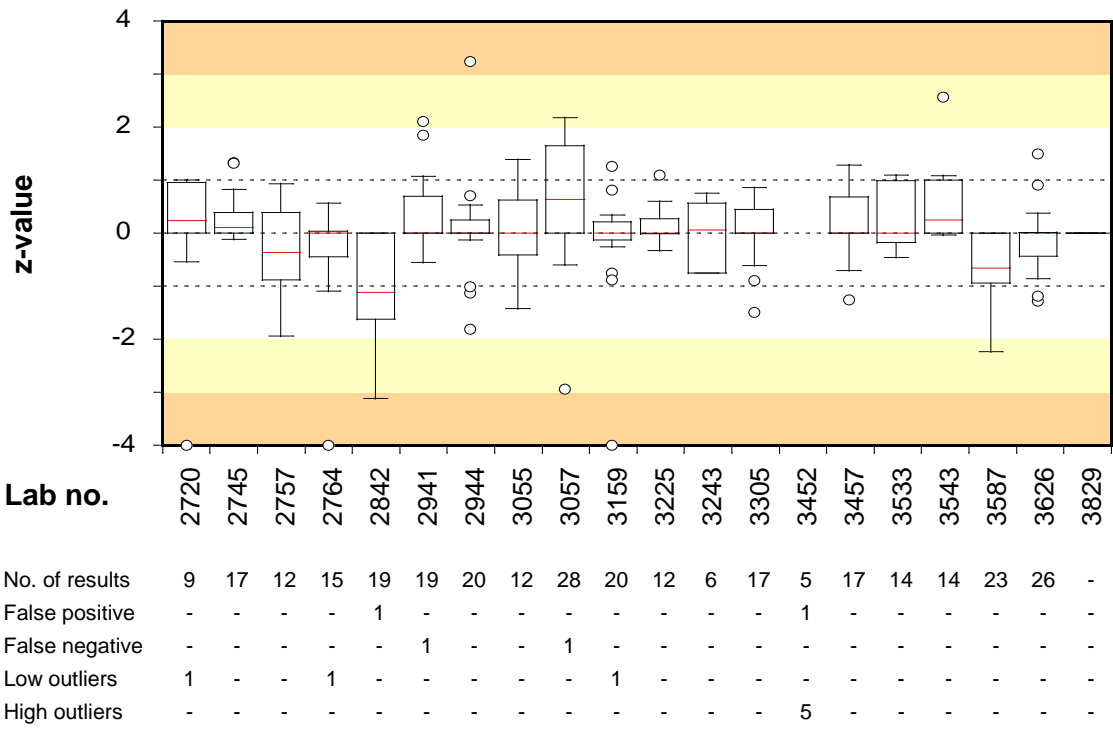
Box plots and numbers of deviating results for each laboratory

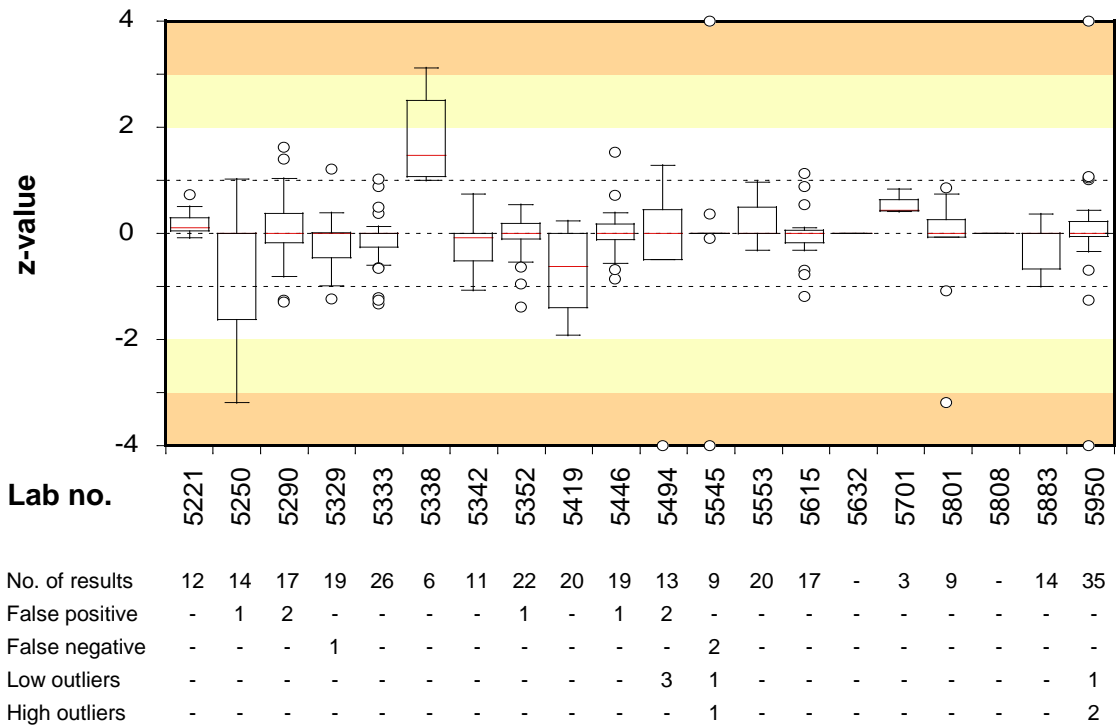
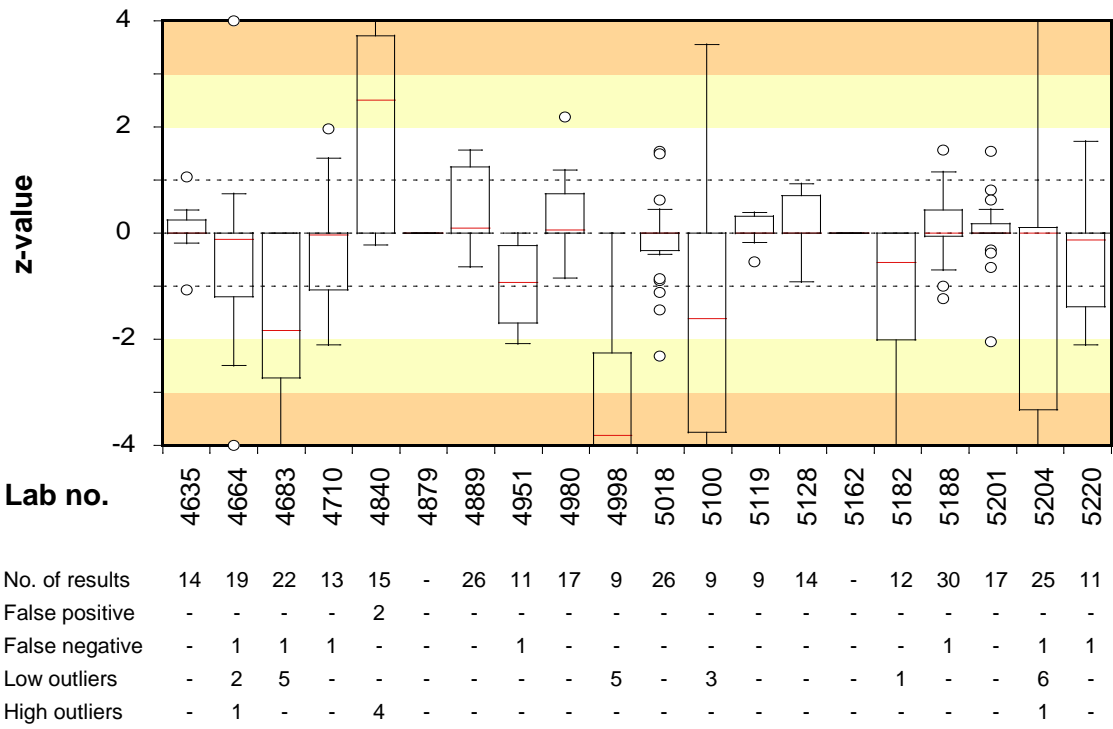
- Z-scores are calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.
- Outliers 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 red line in the box.
- The box includes 50 % of a laboratory’s results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box.
- A circle is for technical reasons shown in the plot when a value deviates to certain degree* from the other values. This does not by itself indicate that the value is an outlier.
- z-scores $>+4$ and <-4 are positioned at $+4$ and -4 , respectively, in the plot.
- The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

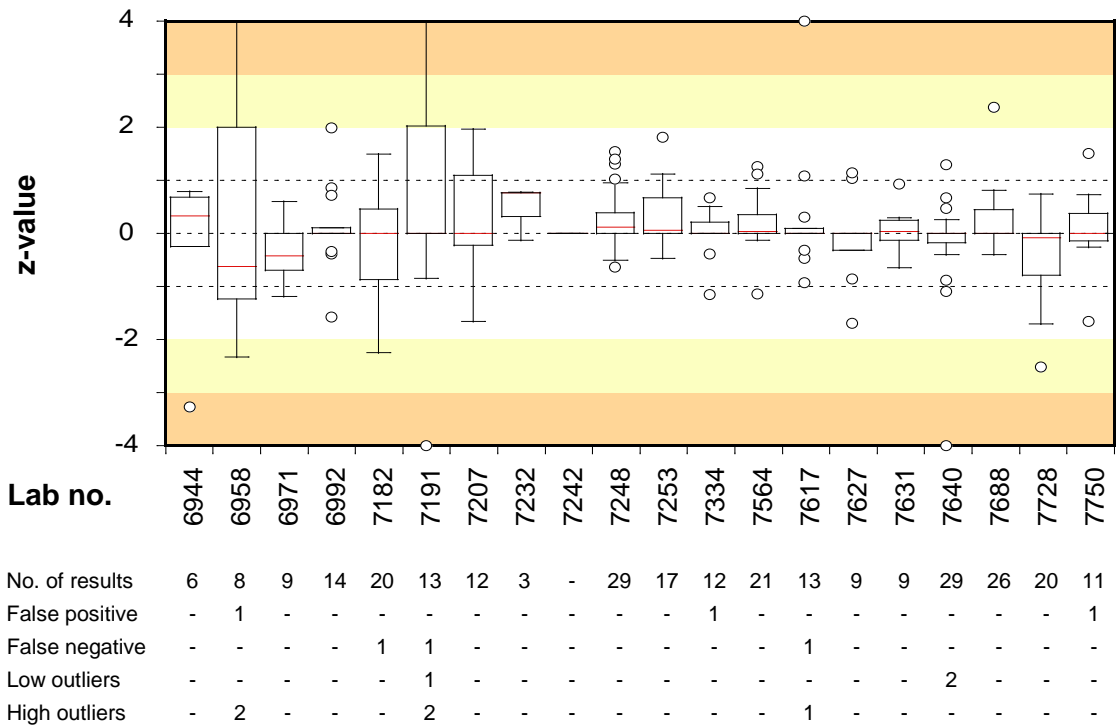
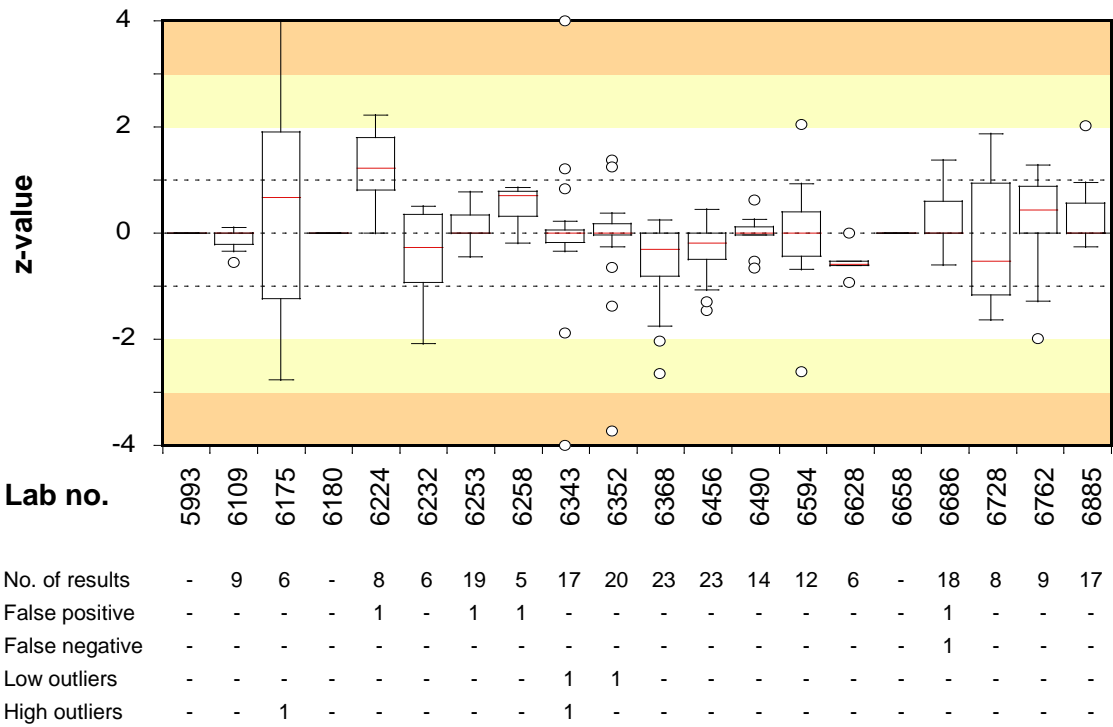
* $< [lowest\ value\ in\ the\ box - 1,5 \times (highest\ value\ in\ the\ box - lowest\ value\ in\ the\ box)]$ or
 $> [highest\ value\ in\ the\ box + 1,5 \times (highest\ value\ in\ the\ box - lowest\ value\ in\ the\ box)]$.

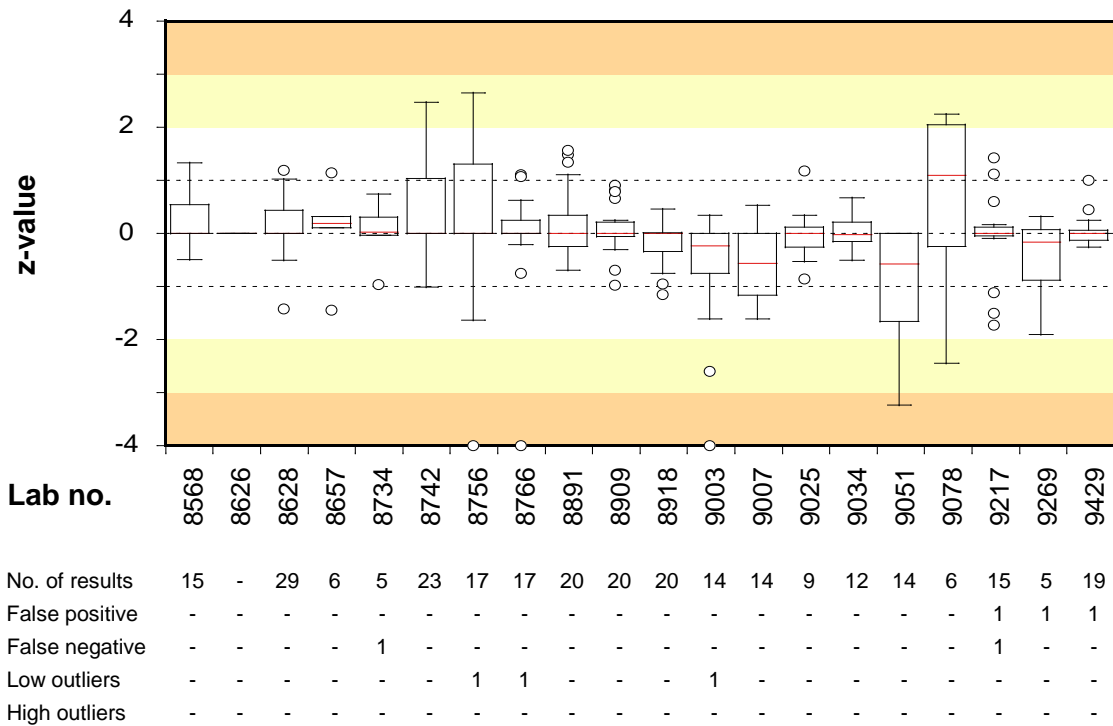
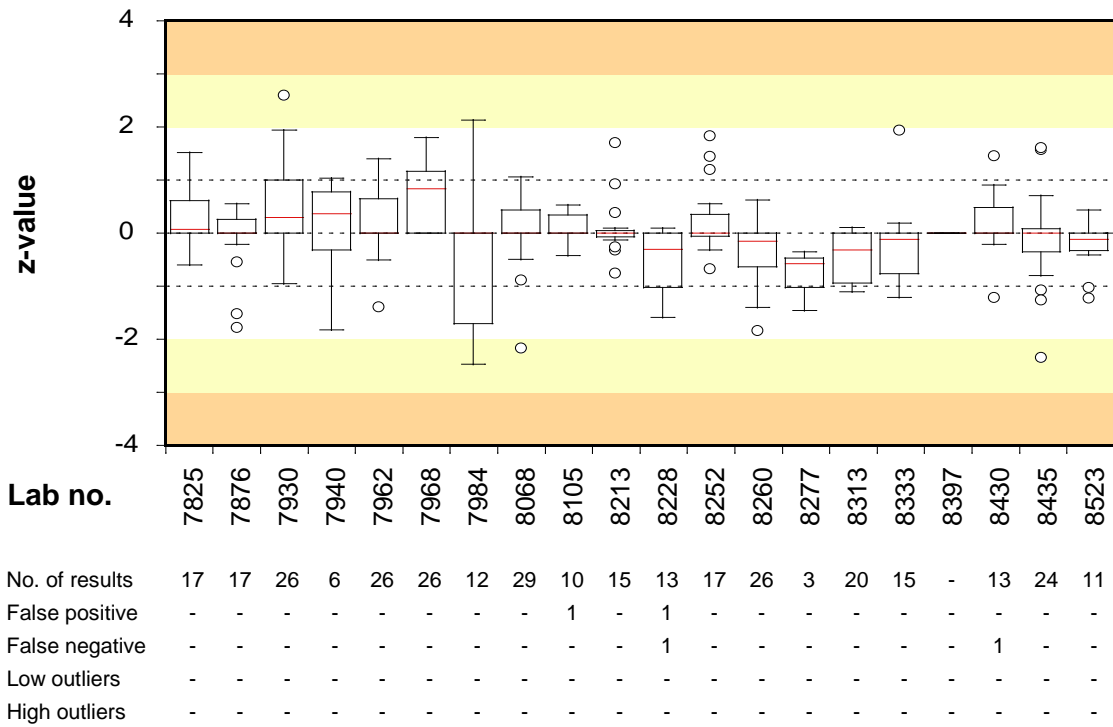


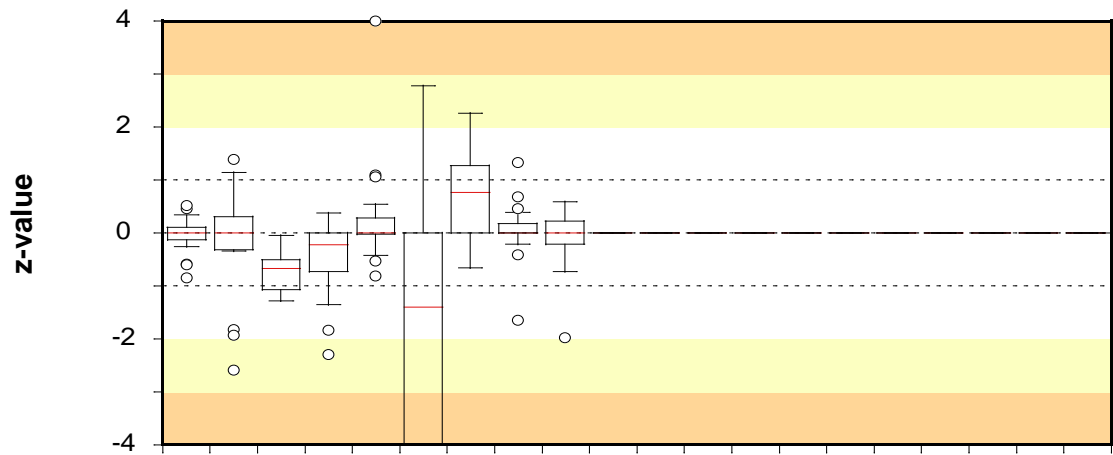
Lab no.	1149	1545	1594	1970	2035	2043	2058	2064	2072	2109	2221	2324	2386	2402	2459	2505	2637	2659	2670	2704
No. of results	14	20	26	29	11	2	9	9	29	9	24	17	14	12	16	15	20	15	14	17
False positive	-	-	-	-	-	1	-	-	-	-	2	-	-	-	1	-	-	2	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-











Lab no.	9436	9453	9512	9559	9662	9747	9890	9903	9950
No. of results	26	17	6	25	23	9	20	17	12
False positive	-	-	-	1	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	3	-	-	-
High outliers	-	-	-	-	1	-	-	-	-

Test material and quality control

Test material

Each laboratory received three manufactured freeze-dried microbial mixtures, 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 had to be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

Table 2. *Microorganisms present in mixtures A-C.*

Mixture ¹	Microorganism	Strain	
		SLV no. ²	Reference ³
A	<i>Enterococcus hirae</i>	SLV-536	CCUG 46536
	<i>Klebsiella pneumoniae</i>	SLV-186	CCUG 45102
	<i>Micrococcus sp.</i>	SLV-055	ATCC 9341
B	<i>Escherichia coli</i>	SLV-477	CCUG 43601
	<i>Serratia marcescens</i>	SLV-040	ATCC 13880
	<i>Staphylococcus hyicus</i>	SLV-546	Chicken
C	<i>Bacillus cereus</i>	SLV-160	CCUG 45098
	<i>Providencia alcalifaciens</i>	SLV-045	CCUG 44809
	<i>Staphylococcus aureus</i>	SLV-350	CCUG 45099

¹ The links between the mixtures and the randomised sample numbers are shown in Annex 1.

² Internal strain identification no. at the National Food Agency.

³ Origin or culture collection (CCUG: Culture Collection University of Gothenburg, Sweden ; ATCC: American Type Culture Collection)

Quality control of the mixtures

It is essential to have aliquots of homogeneous mixture and equal volume in all vials in order to allow comparison of all freeze-dried samples from one mixture. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the mixtures or on 5 vials if an “old” mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a mixture is approved if, for each analysis, the values obtained for the test of reproducibility (T) and the test “Index of dispersion” between vials (I₂) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and I₂, see references 4 and 5 respectively.)

Table 3. Concentration mean (*m*), *T* and *I*₂ values from the quality control of the mixtures; *m* is expressed in log₁₀ cfu (colony forming units) per ml of sample.

Analysis and method	A ¹			B ¹			C ²		
	<i>m</i>	<i>T</i>	<i>I</i> ₂	<i>m</i>	<i>T</i>	<i>I</i> ₂	<i>m</i>	<i>T</i>	<i>I</i> ₂
Aerobic microorganisms 30 °C NMKL method no. 86	4.300	1.16	1.13	4.659	1.29	0.68	4.919	1.61	4.96
Aerobic microorganisms 20 °C NMKL method no. 86	4.287	1.17	1.20	4.597	1.69	2.74	4.948	1.74	5.62
Contaminating microorganisms ISO method no. 13559:2002 IDF method no. 153:2002	4.296	1.87	2.21	4.706	1.27	0.76	5.135	1.33	2.75
Enterobacteriaceae NMKL method no. 144	3.858	1.58	3.37	4.336	1.21	1.01	4.690	1.36	1.21
Coliform bacteria 30 °C NMKL method no. 44	3.822	1.38	1.66	4.168	1.35	1.54	-	-	-
Coliform bacteria. 37 °C NMKL method no. 44	3.853	1.45	2.66	4.200	1.45	2.59	-	-	-
Thermotolerant coliform bacteria NMKL method no. 125	3.845	1.23	0.71	4.234	1.62	0.54	-	-	-
<i>Escherichia coli</i> NMKL method no. 125	-	-	-	4.234	1.62	0.54	-	-	-
Presumptive <i>Bacillus cereus</i> NMKL method no. 67	-	-	-	-	-	-	4.184	1.78	1.14
Coagulase-positive <i>Staphylococci</i> NMKL method no. 66	-	-	-	-	-	-	3.923	1.03	0.02
<i>Enterococci</i> NMKL method no. 68	3.691	1.20	0.42	-	-	-	-	-	-
Gram-negative bacteria in pasteurized milk and cream. Detection of recontamination NMKL method no. 192	Pos.	-	-	Pos.	-	-	Pos.	-	-

- No target organism and therefore no value

¹ n = 10 vials analysed in duplicate

² n = 5 vials analysed in duplicate

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58-64.
2. Anonymous, 2015. Protocol, Microbiology. Drinking Water & Food, The National Food Agency, Sweden.
3. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *J. Appl. Bacteriol.* 74:143-148.
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-Lockefeer, 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 2017

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 nr.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
1149	1 2 3	4.2	4.46	4.83	-	-	-	-	-	-	3.53	3.96	4.79	-	-	-	3.79	3.93	<1	-	-	-	<1	3.93	<1	-	<1	<1	3.68	-	-	-	-	-	-	1149				
1545	2 3 1	4.27	4.84	5.05	-	-	-	-	-	-	3.88	4.55	4.98	-	-	-	-	-	-	3.92	4.51	<1	<1	4.51	<1	<1	<1	4.46	<1	<2	3.96	3.83	<1	<1	-	-	-	1545		
1594	3 2 1	4.28	4.64	5	-	-	-	-	-	-	3.76	4.28	4.9	3.74	4.45	<1	3.84	4.34	<1	3.79	4.34	<1	<1	4.34	<1	<1	<1	4.23	<1	<1	3.82	3.72	<1	<1	-	-	-	1594		
1970	1 3 2	4.02	4.53	4.86	4.03	4.57	4.71	-	-	-	3.6	4.18	4.75	3.72	4.38	<1	3.61	4.48	<1	3.53	4.41	<1	<1	4.41	<1	<1	<1	4.02	<1	<1	3.8	3.65	<1	<1	-	-	-	1970		
2035	3 2 1	4.3	4.7	4.9	-	-	-	-	-	-	3.8	4	4.8	-	-	-	-	-	-	-	-	-	<1	4.1	<1	-	-	-	<1	<1	3.9	-	-	-	-	-	-	2035		
2043	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.7	4.42	4.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2043		
2058	3 2 1	4.3	4.7	4.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	neg	4.35	neg	neg	neg	3.8	-	-	-	-	-	-	-	-	-	2058		
2064	3 1 2	4.21	4.7	4.91	-	-	-	-	-	-	3.9	4.18	4.82	-	-	-	-	-	-	-	-	-	<1	<1	4.47	-	-	-	-	-	-	-	-	-	-	-	-	2064		
2072	3 1 2	4.3	4.58	5.15	4.32	4.61	5.11	-	-	-	3.83	4.11	4.99	3.56	3.85	<1	3.71	4.08	<1	3.78	3.77	<1	<1	2.78	<1	<1	<1	3.74	<1	<1	3.96	3.73	<1	<1	-	-	-	2072		
2109	2 3 1	4.26	4.79	4.89	-	-	-	-	-	-	-	-	-	-	-	-	3.92	4.57	<2	-	-	-	<2	4.24	<2	-	-	-	-	-	-	-	-	-	-	-	-	2109		
2221	1 3 2	5.35	5.98	4.93	-	-	-	3.76	4.49	4.84	3.74	4.3	4.83	3.64	4.25	4.72	3.71	4.28	4.77	-	-	-	<1	4.06	<1	<1	<1	3.8	<1	<1	3.88	3.8	<1	<1	-	-	-	2221		
2324	1 2 3	4.21	4.44	4.89	-	-	-	-	-	-	3.67	4.02	4.91	-	-	-	-	-	-	-	-	-	<1	4.03	<1	<1	<1	4.13	<1	<1	3.8	3.77	<1	<1	-	-	-	2324		
2386	2 1 3	4.2	4.72	5.03	-	-	-	-	-	-	-	-	-	-	-	-	3.87	4.4	<1	3.7	4.26	<1	-	-	-	<1	<1	3.9	<1	4	3.85	-	-	-	-	-	-	2386		
2402	3 2 1	4.26	5.08	5.3	-	-	-	-	-	-	4.18	4.64	4.9	-	-	-	4.04	4.61	<1	-	-	-	<1	4.61	<1	-	-	-	-	-	-	-	-	-	-	-	-	2402		
2459	1 2 3	4.2	4.73	4.89	-	-	-	-	-	-	3.61	4.33	4.84	-	-	-	3.64	4.34	4.85	-	-	-	0	4.32	0	0	0	2.02	0	4.16	3.83	-	-	-	-	-	-	-	2459	
2505	2 3 1	4.28	4.7	4.8	-	-	-	-	-	-	3.68	4.23	4.68	3.66	4.15	<1	3.66	4.18	<1	-	-	-	<1	4.11	<1	-	-	-	-	-	-	-	-	-	-	-	-	2505		
2637	3 1 2	4.23	4.72	4.91	-	-	-	3.38	4.61	4.86	3.69	4.11	4.8	-	-	-	-	-	-	3.56	4.04	<1	<1	4.04	<1	<1	<1	3.7	<1	<1	3.79	-	-	-	-	-	-	2637		
2659	1 3 2	4.23	4.4	4.48	-	-	-	-	-	-	-	-	-	3.84	4.18	4.57	3.84	4.18	4.57	-	-	-	<1	4.23	<1	-	-	-	<1	<1	3.9	-	-	-	-	Pos	Pos	Pos	2659	
2670	1 3 2	4.24	4.85	4.93	-	-	-	-	-	-	-	-	-	-	-	-	4.04	4.04	<1	3.32	4.04	<1	<1	4.04	<1	-	-	-	4.16	3.78	-	-	-	-	-	-	2670			
2704	1 3 2	4.31	4.89	5.12	-	-	-	-	-	-	3.81	4.49	4.81	-	-	-	3.9	4.41	<1	-	-	-	<1	4.41	<1	<1	<1	4.04	<1	<1	3.9	-	-	-	-	-	-	2704		
2720	2 3 1	4.31	4.57	4.94	-	-	-	-	-	-	3.84	4.27	4.93	-	-	-	-	-	-	-	-	-	<1	<1	2.96	-	-	-	-	-	-	-	-	-	-	-	-	-	2720	
2745	2 1 3	4.23	4.63	4.96	-	-	-	-	-	-	3.78	4.45	4.81	-	-	-	-	-	-	3.79	4.19	<1	<1	4.19	<1	<1	<1	4.18	<1	<1	3.98	-	-	-	-	-	-	-	2745	
2757	1 3 2	4.18	4.69	5.04	4	4.3	4.98	-	-	-	3.77	4.11	4.46	3.63	4.08	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2757		
2764	3 1 2	4.18	4.58	4.94	-	-	-	-	-	-	3.66	4.08	4.86	-	-	-	3.71	3.79	<0,60	-	-	-	-	-	-	<1	<1	4	-	-	2.48	<2	<2	-	-	-	-	-	2764	
2842	2 1 3	3.93	4.49	4.48	-	-	-	-	-	-	3.5	3.89	4.58	3.43	4.11	4.62	-	-	-	3.48	3.91	<1	<1	3.93	<1	<1	<1	3.4	<1	<1	3.72	-	-	-	-	-	-	-	2842	
2941	2 3 1	4.41	4.57	4.91	-	-	-	-	-	-	3.86	4.36	4.87	<1	4.28	<1	-	-	-	-	-	-	<1	4.11	<1	<1	<1	4.04	<1	<1	4.04	3.81	<1	<1	-	-	-	-	2941	
2944	1 2 3	4.23	4.72	4.95	-	-	-	-	-	-	3.45	4.17	4.83	-	-	-	3.46	4.15	<1	-	-	-	<1	4.15	<1	<1	<1	4.15	<1	<1	4.2	3.67	<1	<1	-	-	-	-	2944	
3055	2 1 3	4.27	4.79	4.7	-	-	-	-	-	-	3.59	4.46	4.62	-	-	-	-	-	-	-	-	-	<1	<1	4.15	<1	<1	4.15	<1	<1	-	-	-	-	-	Pos	Pos	Pos	3055	
3057	3 1 2	4.38	4.95	4.92	4.2	4.78	5.04	-	-	-	3.29	4.53	4.89	3.92	4.53	<3	3.94	4.49	<3	3.86	4.5	<3	<1	4.5	<1	<1	<1	<1	<1	3.76	3.81	<1	<1	-	-	-	-	3057		
3159	1 3 2	4.15	4.65	4.93	-	-	-	-	-	-	3.75	4.26	4.63	-	-	-	3.96	4.08	<1	3.7	4.15	<1	<1	4.32	<1	<1	<1	4.04	<1	<1	2.52	-	-	-	-	-	-	-	3159	
3225	1 2 3	4.24	4.6	4.88	-	-	-	-	-	-	3.86	4.31	4.82	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	3.97	-	-	-	-	-	-	-	Pos	Pos	Pos	3225	
3243	3 1 2	4.15	4.54	4.93	-	-	-	-	-	-	3.7	4.34	4.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3243		
3305	1 2 3	4.3	4.56	4.87	-	-	-	-	-	-	3.58	4.2	4.9	-	-	-	-	-	-	3.73	4.3	<1	<1	4.3	<1	<1	<1	3.6	<1	<1	3.88	-	-	-	-	-	-	-	3305	
3452	2 3 1	5.07	5.65	5.53	-	-	-	-	-	-	-	-	-	4.77	5.08	4.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3452		
3457	2 3 1	-	-	-	4.06	4.56	4.9	-	-	-	3.75	4.4	4.88	-	-	-	3.8	4.42	<1	-	-	-	<1	4.42	<1	-	-	-	4.32	3.95	3.65	<1	<1	-	-	-	-	3457		
3533	1 2 3	4.320	4.720	4.840	-	-	-	-	-	-	-	-	-	-	-	-	3.66	4	<1	3.66	4	<1	<1	4	<1	-	-	-	<1	3.790	-	-	-	-	-	-	-	3533		
3543	2 3 1	4.320	4.640	5.040	-	-	-	-	-	-	3.74	4.320	5.180	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	4.04	<1	<1	3.950	3.830	<1	<1	-	-	-	-	3543	
3587	1 2 3	4.1	4.56	4.75	-	-	-	-	-	-	3.4	4.07	4.7	3.4	4.04	<1	3.53	4.06	<1	-	-	-	<1	4	<1	<1	<1	3.6	<1	<1	3.7	3.7	<1	<1	-	-	-	-	3587	
3626	2 1 3	4.10	4.6	4.9	-	-	-	-	-	-	3.6	4.2	4.70	3.5	4.2	<1	3.6	4.2	<1	3.6	4.2	<1	<1	4.2	<1	<1	<1	4.2	<1	<1	4	3.7	<1	<1	-	-	-	-	3626	
3829	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3829	
3831	1 3 2	4.29	4.47	4.99	4.32	4.26	5.03	-	-	-	-	-	-	-	-	-	3.72	3.18	0	-	-	-	0	3.84	0	-	-	-	-	-	-	-	-	-	-	-	-	-	3831	
3864	2 3 1	-	-	-	-	-	-	-	-	-	3.58	4.13	4.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Pos	3864
3868	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3868	
m		4.219	4.646	4.905	4.140	4.497	4.911	4.054	4.519	4.879	3.706	4.195	4.770	3.675	4.1																									

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 nr.	
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
7688	3	2	1	4.22	4.59	4.89	-	-	-	-	-	-	3.7	4.23	4.74	3.77	4.28	0	3.66	4.15	0	3.8	4.32	0	0	4.32	0	0	0	4	0	0	3.88	3.94	0	0	-	-	-	7688	
7728	3	1	2	4.15	4.75	4.79	4.04	4.51	4.82	-	-	-	-	-	-	-	-	-	3.66	3.88	<1	3.66	3.88	<1	<1	3.88	<1	<1	<1	4.11	<1	<1	4.11	<1	<1	3.54	-	-	-	7728	
7750	1	3	2	4.36	4.61	4.95	-	-	-	-	-	-	3.67	4.22	4.84	-	-	-	3.69	4.3	4.75	-	-	-	-	-	-	<1	<1	3.56	-	-	-	-	-	-	-	7750			
7825	3	2	1	4.36	4.73	5.07	-	-	-	-	-	-	3.8	4.26	4.91	-	-	-	-	-	-	3.64	4.29	<1	<1	4.09	<1	-	-	<1	<1	3.85	3.76	<1	<1	-	-	-	7825		
7876	3	2	1	4.2	4.57	4.65	-	-	-	-	-	-	3.77	4.3	4.79	-	-	-	-	-	-	-	-	-	-	<1	<1	4.04	<1	<1	3.89	3.63	<1	<1	-	-	-	-	7876		
7930	3	1	2	4.27	4.75	4.94	-	-	-	-	-	-	3.98	4.26	4.88	4.06	4.4	<1	4	4.49	<1	4	4.36	<1	<1	4.36	<1	<1	<1	4.17	<1	<1	3.72	3.75	<1	<1	-	-	-	7930	
7940	1	2	3	4.19	4.79	5.01	-	-	-	-	-	-	-	-	-	3.79	3.85	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7940		
7962	2	1	3	4.35	4.75	4.98	-	-	-	-	-	-	3.65	4.32	4.8	3.78	4.36	0	3.77	4.3	0	3.83	4.2	0	0	4.2	0	0	0	3.92	0	0	3.77	3.64	0	0	-	-	-	7962	
7968	3	2	1	4.28	4.86	5.07	-	-	-	-	-	-	3.83	4.36	4.95	3.81	4.32	<1	3.94	4.43	<1	3.86	4.53	<1	<1	4.53	<1	<1	<1	4.34	<1	<1	3.87	3.89	<1	<1	-	-	-	7968	
7984	1	3	2	4	4.3	4.9	-	-	-	-	-	-	3.48	4.6	4.48	-	-	-	-	-	-	-	-	-	-	<1	<1	4	-	-	-	-	-	-	-	Pos	Pos	Pos	7984		
8068	1	3	2	4.26	4.6	4.85	4.26	4.58	4.88	-	-	-	3.83	4.2	4.63	3.76	3.79	0	3.88	4.2	0	3.84	4.26	0	0	4.26	0	0	0	3.85	0	0	3.83	3.76	0	0	-	-	-	8068	
8105	2	1	3	4.18	4.72	4.96	-	-	-	-	-	-	-	-	-	-	-	-	3.74	4.19	4.73	-	-	-	0	4.14	0	-	-	-	-	4.3	3.83	-	-	-	-	-	-	8105	
8213	1	2	3	4.19	4.61	5.04	-	-	-	-	-	-	3.72	4.17	4.65	-	-	-	-	-	-	-	-	-	<1	4.23	<1	<1	<1	4.4	-	-	-	-	-	-	Pos	Pos	Pos	8213	
8228	2	1	3	4.21	4.46	4.79	3.96	4.32	4.79	-	-	-	-	-	-	3.65	4.12	neg	neg	4.12	4.62	-	-	-	-	-	-	neg	neg	3.72	-	-	-	-	-	-	-	8228			
8252	3	2	1	4.23	4.81	5.11	-	-	-	-	-	-	3.76	4.15	4.76	-	-	-	3.56	4.08	<1	-	-	-	<1	4.08	<1	<1	<1	4.11	<1	<1	4.04	-	-	-	-	-	8252		
8260	1	3	2	4.09	4.6	4.76	-	-	-	-	-	-	3.51	4.07	4.7	3.59	4.1	<1	3.51	4.1	<1	3.45	4.08	<1	<1	4.08	<1	<1	<1	4.13	<1	<1	3.76	3.76	<1	<1	-	-	-	8260	
8277	2	3	1	-	-	-	-	-	-	3.64	4.45	4.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8277		
8313	3	2	1	4.16	4.57	4.77	-	-	-	-	-	-	3.55	4.1	4.69	3.53	3.98	<1	-	-	-	-	-	-	<1	3.93	<1	<1	<1	4	<1	<1	3.72	3.74	<1	<1	-	-	-	8313	
8333	2	1	3	4.4	4.62	4.75	-	-	-	-	-	-	3.69	3.98	4.8	-	-	-	3.54	3.88	<0,60	-	-	-	-	-	-	<2	<2	3.67	-	-	-	3.7	<2	<2	-	-	-	8333	
8397	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8397		
8430	1	2	3	4.2	4.85	4.97	-	-	-	-	-	-	3.72	4.3	<1	3.67	4.26	<1	-	-	-	-	-	-	<1	4.34	<1	-	-	<1	4.45	3.69	-	-	-	-	-	-	8430		
8435	3	2	1	-	-	4.79	4.16	4.43	4.63	-	-	-	3.69	-	4.6	3.49	-	<1	3.6	4.28	<1	3.72	4.49	<1	<1	4.49	<1	<1	-	4.15	<1	<1	3.85	3.73	<1	<1	-	-	-	8435	
8523	3	2	1	4.2	4.63	4.73	-	-	-	-	-	-	3.74	4	4.84	-	-	-	-	-	-	-	-	-	<1	4.06	<1	-	-	<1	<1	3.8	-	-	-	-	-	-	-	8523	
8568	3	1	2	4.3	4.68	4.88	-	-	-	-	-	-	3.86	4.45	4.71	-	-	-	3.79	4.27	<0,60	-	-	-	-	-	-	<1	<1	3.85	-	-	-	3.77	<2	<2	-	-	-	8568	
8626	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8626		
8628	2	1	3	4.33	4.6	4.9	4.24	4.28	5.01	-	-	-	3.85	4.19	4.85	3.79	4.25	<1	3.9	4.1	<1	3.79	4.23	<1	<1	4.23	<1	<1	<1	4.05	<1	<1	3.77	3.74	<1	<1	-	-	-	8628	
8657	3	1	2	4.23	4.69	5.07	-	-	-	-	-	-	3.74	4.22	4.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8657		
8734	3	1	2	4.13	4.75	4.9	-	-	-	-	-	-	3.75	4.2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8734		
8742	1	2	3	4.2	4.54	4.76	-	-	-	-	-	-	3.74	4.11	4.77	4.04	4.38	<1	4.04	4.38	<1	4.04	4.38	<1	<1	4.38	<1	<1	<1	3.88	<1	<1	3.95	-	-	-	-	-	-	8742	
8756	2	1	3	4.2	4.83	5.11	-	-	-	-	-	-	4.08	4.41	5	-	-	-	-	-	-	-	-	-	<1	4.16	<1	<1	<1	4.3	<1	<1	3.36	3.62	<1	<1	-	-	-	8756	
8766	2	3	1	4.2	4.8	4.3	-	-	-	-	-	-	3.6	4.4	4.8	-	-	-	-	-	-	-	-	-	<1	4.2	<1	<1	<1	4	<1	<1	3.9	3.8	<1	<1	-	-	-	8766	
8891	2	3	1	4.17	4.61	4.95	-	-	-	4.47	4.74	5.04	3.67	4.18	4.83	3.66	4.05	<1	-	-	-	-	-	-	<1	4.06	<1	<1	<1	4.31	<1	<1	4.3	3.8	-	-	-	-	-	8891	
8909	2	3	1	4.21	4.67	5	-	-	-	-	-	-	3.82	4.17	4.78	3.81	4.12	<1	-	-	-	-	-	-	<1	4	<1	<1	<1	3.73	<1	<1	3.92	3.77	<1	<1	-	-	-	8909	
8918	3	2	1	4.2	4.71	4.85	-	-	-	4.12	4.54	4.76	3.73	4.15	4.65	-	-	-	3.7	3.95	<1	-	-	-	<1	4.15	<1	<1	<1	3.9	<1	<1	3.72	-	-	-	-	-	-	8918	
9003	2	1	3	4.2	4.61	4.82	-	-	-	-	-	-	3.6	3.89	3.64	-	-	-	3.59	4.19	<1	-	-	-	<1	4.19	<1	-	-	-	4.4	3.53	-	-	-	-	-	-	9003		
9007	1	3	2	4.07	4.72	4.97	-	-	-	-	-	-	3.58	4.15	4.58	-	-	-	3.48	3.72	0	-	-	-	0	3.9	0	-	-	-	4.26	3.72	-	-	-	-	-	-	9007		
9025	3	1	2	4.14	4.81	4.83	-	-	-	-	-	-	3.67	4.26	4.79	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Pos	9025		
9034	1	3	2	4.2	4.6	4.9	4.2	4.6	4.9	-	-	-	3.7	4.1	4.8	-	-	-	-	-	-	-	-	-	<1	4.2	<1	-	-	-	-	-	-	-	-	-	-	-	-	9034	
9051	3	2	1	4.17	4.38	4.64	-	-	-	-	-	-	3.62	3.88	4.59	-	-	-	-	-	-	-	-	-	<1	3.46	<1	<1	<1	3.94	<1	<1	3.66	-	-	-	-	-	-	-	9051
9078	1	3	2	4.410	4.960	4.870	-	-	-	-	-	-	3.82	4.460	4.380	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078		
9217	3	1	2	4.080	4.490	5.110	-	-	-	-	-	-	3.73	4.310	4.780	-	-	-	-	-	-	-	-	-	-	3.71	<1	<1	<1	<1	3.95	<1	4.21	3.630	3.840	<2	<2	-	-	-	9217
9269	3	2	1	4.2	4.38	4.78	-	-	-	-	-	-	3.72	4.2	4.81	-	-	-	3.76	4.11	3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9269		
9429	2	3	1	4.2	4.62	4.94	-	-	-	-	-	-	3.72	4.2	4.81	-	-	-	3.68	4.08	4.61	3.7	4.15	<1	<1	4.11	<1	-	-	<1	<1	3.88	3.83	<1	<1	-	-	-	-	9429	
9436	3	2	1	4.24	4.71	4.92	-	-	-	-	-	-	3.71	4.26	4.73																										

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			<i>Escherichia coli</i>			Presumptive <i>Bacillus cereus</i>			Coagulase-positive Staphylococci			Enterococci			Gram-neg bacteria in dairy prod.			Lab nr.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
9662	2	3	1	4.18	4.64	4.83	-	-	-	-	-	-	3.86	4.22	4.83	4.84	4.16	<1	3.81	4.22	<1	-	-	-	<1	4.19	<1	<1	<1	3.77	<1	<1	3.95	3.72	<1	<1	-	-	-	9662
9747	1	3	2	4.14	4.45	4.51	-	-	-	-	-	-	3.11	3.04	3.99	-	-	-	-	-	-	-	-	-	<1	<1	4.67	-	-	-	-	-	-	-	-	-	-	-	-	9747
9890	3	1	2	4.43	4.74	5.03	4.15	4.7	5.08	-	-	-	3.88	4.45	4.98	-	-	-	3.94	4.34	0	-	-	-	0	4.34	0	0	0	3.81	0	0	3.82	-	-	-	-	-	-	9890
9903	2	1	3	4.2	4.71	4.93	-	-	-	-	-	-	3.72	4.27	4.88	-	-	-	-	-	-	-	-	-	<1	4.43	<1	<1	<1	3.87	<1	<1	3.64	3.75	<1	<1	-	-	-	9903
9950	1	3	2	4.22	4.66	4.8	-	-	-	4.22	4.55	4.91	-	-	-	-	-	-	-	-	-	3.78	4.12	<1	-	-	-	<1	<1	3.48	-	-	-	-	-	-	-	-	-	9950

N		167	167	168	28	28	28	17	17	17	142	140	142	55	54	55	93	93	91	47	47	47	115	113	115	117	116	117	107	106	107	72	71	71	11	11	11	N	
Min		0	3.72	3.7	3.91	4.26	4.63	2.7	2.48	2.98	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	Min
Max		5.35	5.98	5.53	4.32	4.80	5.11	4.47	4.75	5.04	5.03	5.03	5.28	4.84	5.08	4.97	4.44	4.61	4.85	4.04	4.53	4.71	3.71	5.02	0	3.90	4.23	4.67	0	4.45	4.94	4.74	4.50	0	-	-	-	Max	
Med		4.21	4.64	4.92	4.17	4.49	4.91	4.12	4.55	4.88	3.72	4.20	4.79	3.65	4.18	0	3.69	4.12	0	3.75	4.20	0	0	4.17	0	0	0	4.00	0	0	3.84	3.76	0	0	-	-	-	Med	
m		4.219	4.646	4.905	4.140	4.497	4.911	4.054	4.519	4.879	3.706	4.195	4.770	3.675	4.173	0	3.696	4.094	0	3.730	4.197	0	0	4.147	0	0	0	3.974	0	0	3.829	3.751	0	0	pos	pos	pos	m	
s		0.093	0.140	0.144	0.113	0.153	0.120	0.281	0.201	0.103	0.141	0.190	0.160	0.148	0.177	0	0.209	0.280	0	0.153	0.185	0	0	0.213	0	0	0	0.250	0	0	0.115	0.080	0	0	-	-	-	s	
F+		0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	15	0	0	2	3	0	0	2	2	0	0	17	0	0	1	0	0	0	0	0	F+
F-		1	0	0	0	0	0	0	0	0	3	1	4	1	1	0	1	2	0	2	0	0	0	2	0	0	0	0	4	0	0	2	1	0	0	0	0	0	F-
<		4	2	3	0	0	0	1	1	1	3	4	5	1	2	0	0	0	0	0	2	0	0	6	0	0	0	0	4	0	0	6	3	0	0	-	-	-	<
>		6	3	1	0	0	0	0	0	0	2	1	0	2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	6	0	0	-	-	-	>	
< OK		3.93	4.20	4.47	3.91	4.26	4.63	3.38	4.00	4.68	3.25	3.68	4.31	3.40	3.69	0	2.97	3.18	0	3.32	3.77	0	0	3.46	0	0	0	3.32	0	0	3.53	3.54	0	0	-	-	-	< OK	
> OK		4.54	5.08	5.30	4.32	4.80	5.11	4.47	4.75	5.04	4.08	4.64	5.28	4.06	4.53	0	4.44	4.61	0	4.04	4.53	0	0	4.61	0	0	0	4.67	0	0	4.20	3.94	0	0	-	-	-	> OK	

N = number of analyses performed
Min = lowest reported result

Max = highest reported result
Median = median value

m = mean value
s = standard deviation

F+ = false positive
F- = false negative

< = low outlier
> = high outlier

< OK = lowest accepted value
> OK = highest accepted value

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 National Food Agency's PT program offers

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

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

The National Food Agency's reference material

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

More information is available on our website: www.livsmedelsverket.se/en/RM-micro