

Food Microbiology

October 2016

Jonas Ilbäck



Edition

Version 1 (2016-12-30)

Editor in chief

Hans Lindmark, head of Biology department, National Food Agency

Responsible for the scheme

Jonas Ilbäck, microbiologist, Biology department, National Food Agency

PT October 2016 is registered as no. 2016/02332 at the National Food Agency.

Proficiency Testing

Microbiology – Food

October 2016



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
BAA	Bile Aesculin 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
EMB	Eosin Methylene Blue agar
ENT	Slanetz & Bartley <i>Enterococcus</i> agar
KAAA	Kanamycin Aesculin Azid Agar
LSB	Laurul 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 2016	5
- General outcome	5
- Aerobic microorganisms, 20 °C and 30 °C	6
- Contaminating microorganisms in dairy products.....	8
- Enterobacteriaceae	10
- Coliform bacteria 30 °C and 37 °C.....	12
- Thermotolerant coliform bacteria and <i>Escherichia coli</i> ..	14
- Presumptive <i>Bacillus cereus</i>	17
- Coagulase positive staphylococci	18
- Enterococci	20
- Gram-negative bacteria in pasteurized dairy products	22
Outcome of the results of individual laboratory – assessment	24
- Box plot	25
Test material and quality control	31
- Test material	31
- Quality control of the mixtures	32
References	33
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 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). All reported results are presented in Annex 1.



According to EN ISO/IEC 17043, for which the proficiency testing programme organised by the National Food Agency is accredited since early 2012, it is mandatory for the participating laboratories to give method information for all analyses for which they report results. Method information is sometimes difficult to interpret, e.g. since many laboratories choose a medium that differs from that in the reported standard methods. Results from laboratories that have reported ambiguous 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.

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

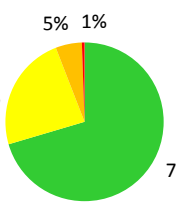
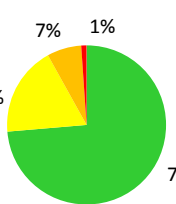
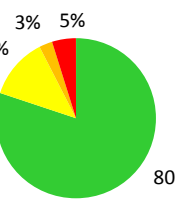
Results of the PT round October 2016

General outcome

Samples were sent to 193 laboratories, 49 in Sweden, 124 in other European countries, and 20 outside Europe. Of the 186 laboratories that reported results, 93 (50 %) provided at least one result that received an annotation. In the previous round with similar analyses (October 2015), the proportion was 48 %.

Individual results for each analysis of 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			
% of participants with													
		0 annotation 1 annotations 2 annotations >2 annotations											
Organisms		<i>Bacillus cereus</i> <i>Pediococcus acidilactici</i> <i>Staphylococcus xylosus</i>				<i>Enterobacter aerogenes</i> <i>Enterococcus durans</i> <i>Proteus mirabilis</i>				<i>Staphylococcus saprophyticus</i> <i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Enterococcus faecium</i>			
Analysis		Target organism	N	F%	X%	Target organism	N	F%	X%	Target organism	N	F%	X%
Aerobic mikroorg.	30 °C	All	176	0	3	All	176	1	6	All	175	0	6
	20 °C		32	0	13		32	0	6		32	0	6
Contaminating microorganisms		All	17	0	12	All	17	0	0	All	17	0	6
Enterobacteriaceae		-	143	1	0	<i>E. aerogenes</i> <i>P. mirabilis</i>	145	1	1	<i>E. coli</i>	145	1	3
Coliform bacteria	30 °C	-	57	2	0	<i>E. aerogenes</i> *	57*	11*	5*	<i>E. coli</i>	56	4	0
	37 °C		100	1	0		100*	8*	7*		100	3	7
Thermotolerant coliform bacteria		-	51	2	0	(<i>E. aerogenes</i>)	51	20	0	<i>E. coli</i>	51	4	4
<i>E. coli</i>		-	125	2	0	-	125	2	0	<i>E. coli</i>	125	2	5
Presumptive <i>B. cereus</i>		<i>B. cereus</i>	122	2	2	-	119	4	0	-	119	3	0
Coagulase-positive staphylococci		(<i>S. xylosus</i>)	120	12	0	-	117	2	0	<i>S. aureus</i>	120	3	8
Enterococci		(<i>P. acidilactici</i>)**	76**	42**	0**	<i>E. durans</i>	75	5	4	<i>E. faecium</i>	76	1	8
Gram-negative bacteria in dairy products		-	12	8	-	<i>E. aerogenes</i> <i>P. mirabilis</i>	12	0	-	<i>E. coli</i>	12	0	-

- : no target organism or no value

(microorganism): false positive before confirmation

* Negative results are also considered as correct for this analysis

** Positive results are also considered as correct for this analysis

Aerobic microorganisms, 20 °C and 30 °C

Mixture A

All microorganisms in mixture A were target organisms for the analysis. Most colonies at both temperatures consisted of a strain of *Staphylococcus xylosus*, since that was present in the largest concentration in the mixture. The results were distributed with a distinct peak, and only a few outliers were reported.

Mixture B

All microorganisms in mixture B were target organisms for the analysis. A strain of *Enterococcus durans* was present in the highest concentration, and thus most colonies at both 20 °C and 30 °C were from this species. The results were distributed well, with a small number of outliers.

Mixture C

All microorganisms in mixture C were target organisms for the analysis. Strains of *Staphylococcus aureus* and *E. coli* were present in the highest concentrations, and thus constituted most of the colonies. As for mixtures A and B, the results were distributed around a distinct peak, and a small number of outliers were reported.

General remarks

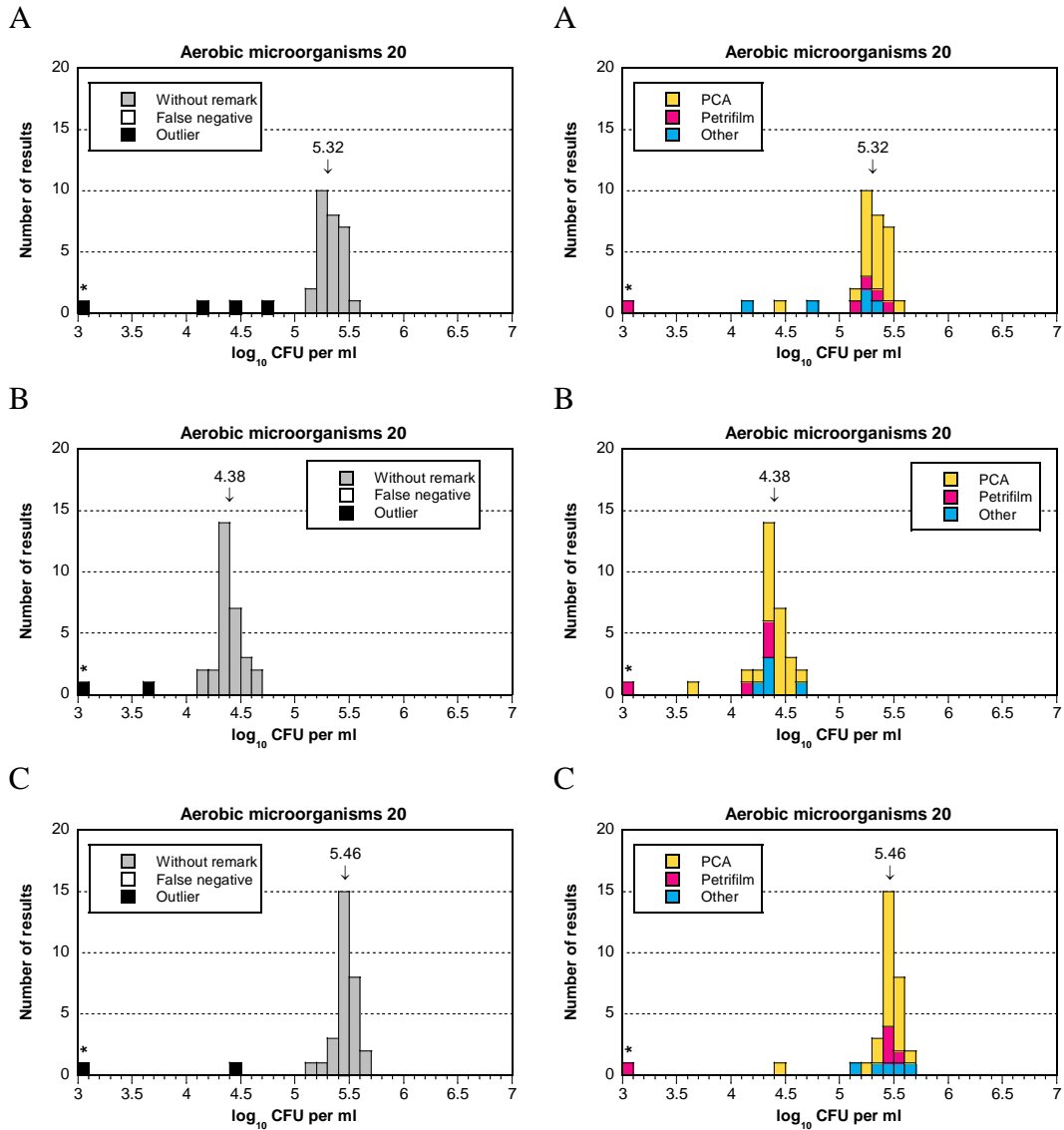
As a whole, the analyses of aerobic microorganisms were unproblematic for the laboratories, and no differences based on the use of a specific method or media were observed.

Most laboratories followed either NMKL 86 or ISO 4833, both of which prescribe incubation on PCA for 72 hours. As an alternative, ISO 4833 allows using MPCA as a substitute for PCA in the analysis of samples from milk or milk products. Two laboratories specified that they followed NMKL 86:1999, which has been replaced by both NMKL 86:2006 and NMKL 86:2013. Regardless of the choice of method and media, equivalent results were reported for all three mixtures.

As in the proficiency testing round October 2015, approximately 20 % of the laboratories used Petrifilm™ Aerobic count (Petrifilm AC), with results no different compared to NMKL 86 and ISO 4833. In the analysis performed at 30 °C, 5 laboratories used TEMPO® (bioMérieux® SA, Marcy l'Etoile, France), either TEMPO® AC or TEMPO® TVC. These systems are based on the hydrolysis of a fluorescent indicator in the media by the microorganisms. The sample is incubated in a card that contains wells with different volumes, and the concentration measurement is based on MPN (Most Probable Number) and determined by the emitted fluorescence. Users of TEMPO® reported slightly higher results compared to other methods, for all three mixtures, however the number of laboratories that used TEMPO® is too low to evaluate this observation further.

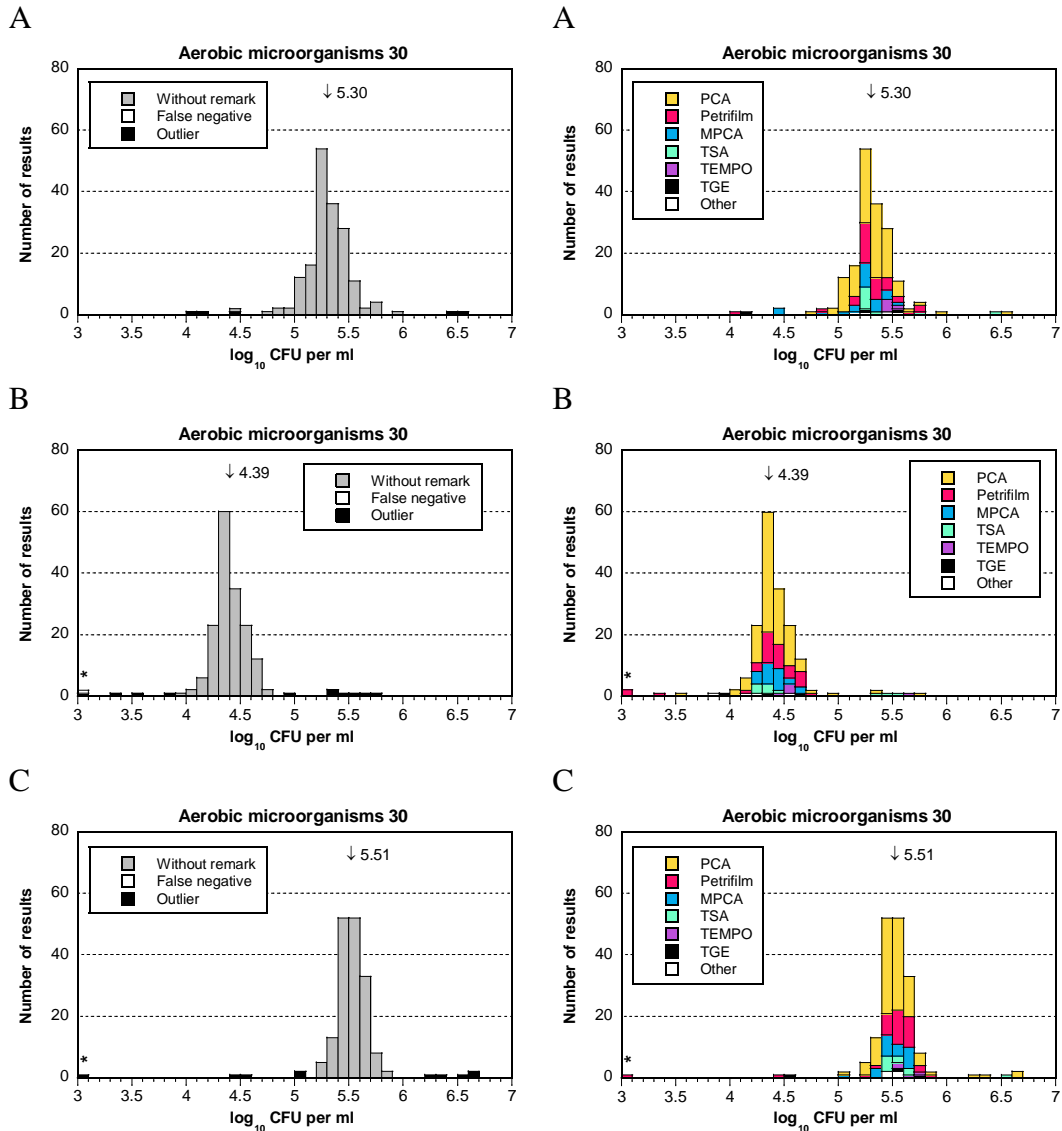
Results from analysis of aerobic microorganisms, 20 °C

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	< >	n	m	S	F	< >	n	m	s	F	< >			
All results	32	28	5.32	0.10	0	4	0	30	4.38	0.12	0	2	0	30	5.46	0.11	0	2	0
PCA	22	21	5.33	0.10	0	1	0	21	4.40	0.12	0	1	0	21	5.46	0.09	0	1	0
Petrifilm AC	5	4	-	-	0	1	0	4	-	-	0	1	0	4	-	-	0	1	0
Other	5	3	-	-	0	2	0	5	4.39	0.15	0	0	0	5	5.43	0.20	0	0	0



Results from analysis of aerobic microorganisms, 30 °C

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
All results	176	170	5.30	0.18	0	4	2	164	4.39	0.13	1	4	7	165	5.51	0.11	0	5	5
PCA	97	96	5.29	0.17	0	0	1	93	4.38	0.13	0	1	3	92	5.50	0.11	0	1	4
Petrifilm AC	35	33	5.33	0.17	0	2	0	32	4.43	0.13	1	2	0	33	5.55	0.12	0	2	0
MPCA	22	21	5.23	0.23	0	1	0	22	4.40	0.11	0	0	0	21	5.51	0.11	0	1	0
TSA	10	9	5.32	0.18	0	0	1	7	4.31	0.11	0	0	3	9	5.50	0.08	0	0	1
TEMPO	5	5	5.44	0.05	0	0	0	4	-	-	0	0	1	4	-	-	0	0	0
TGE	3	2	-	-	0	1	0	3	-	-	0	0	0	2	-	-	0	1	0
Other	4	4	-	-	0	0	0	3	-	-	0	1	0	4	-	-	0	0	0



Contaminating microorganisms in dairy products

Mixture A

Mixture A contained strains of *Pedicoccus acidilactici*, *Staphylococcus xylosus* and *Bacillus cereus*. The strain of *S. xylosus* was present in the highest concentration, and thus most colonies were from this species. The majority of the results were distributed around a concentration corresponding to that of *S. xylosus* in the mixture, though two laboratories reported results that were considerably lower.

Mixture B

Mixture B contained strains of *Enterobacter aerogenes*, *Proteus mirabilis* and *Enterococcus durans*. The strain of *E. durans* was present in the highest concentration, and thus most colonies were from this species. The majority of the reported results were also distributed around a concentration corresponding to that of *E. durans* in the mixture.

Mixture C

Mixture C contained strains of *Staphylococcus saprophyticus*, *S. aureus*, *Escherichia coli* and *Enterococcus faecium*. The strains of *S. aureus* and *E. coli* were present in the highest concentrations, and thus most colonies were from these two species. The majority of the laboratories also reported results corresponding to those of *S. aureus* and *E. coli* in the mixture. One laboratory reported a result that was considerably lower.

General remarks

Only 17 laboratories performed the analysis, of which 12 (71 %) specified that they followed the standard ISO 13559:2002 / IDF 153:2002. All laboratories stated the use of sugar free agar (SFA) as media. The low number of participants makes statistical evaluation of the results difficult and mean values are therefore given in tables in figures. Still, the results for all three mixtures have a more narrow distribution compared to PT rounds previous years (2013-2015), and the results also fit well with the concentrations measured at the National Food Agency (Table 3).

The aim of the analysis is to identify potential contaminating microorganisms in dairy products. Lactic acid bacteria, which are catalase negative, are not to be included and thus several laboratories perform a catalase test to determine which colonies to count. Such a test is however not a part of ISO 13559:2002 / IDF 153:2002, which only requirement is the counting of colonies that are characteristic contaminating microorganisms. Though the strain of *E. durans* present in the highest concentration in mixture B is catalase negative, both high and low concentrations were reported, regardless of whether a catalase test was performed or not. Possibly, the presence of swarming *Proteus* could have had an effect on the counting of colonies in this mixture.

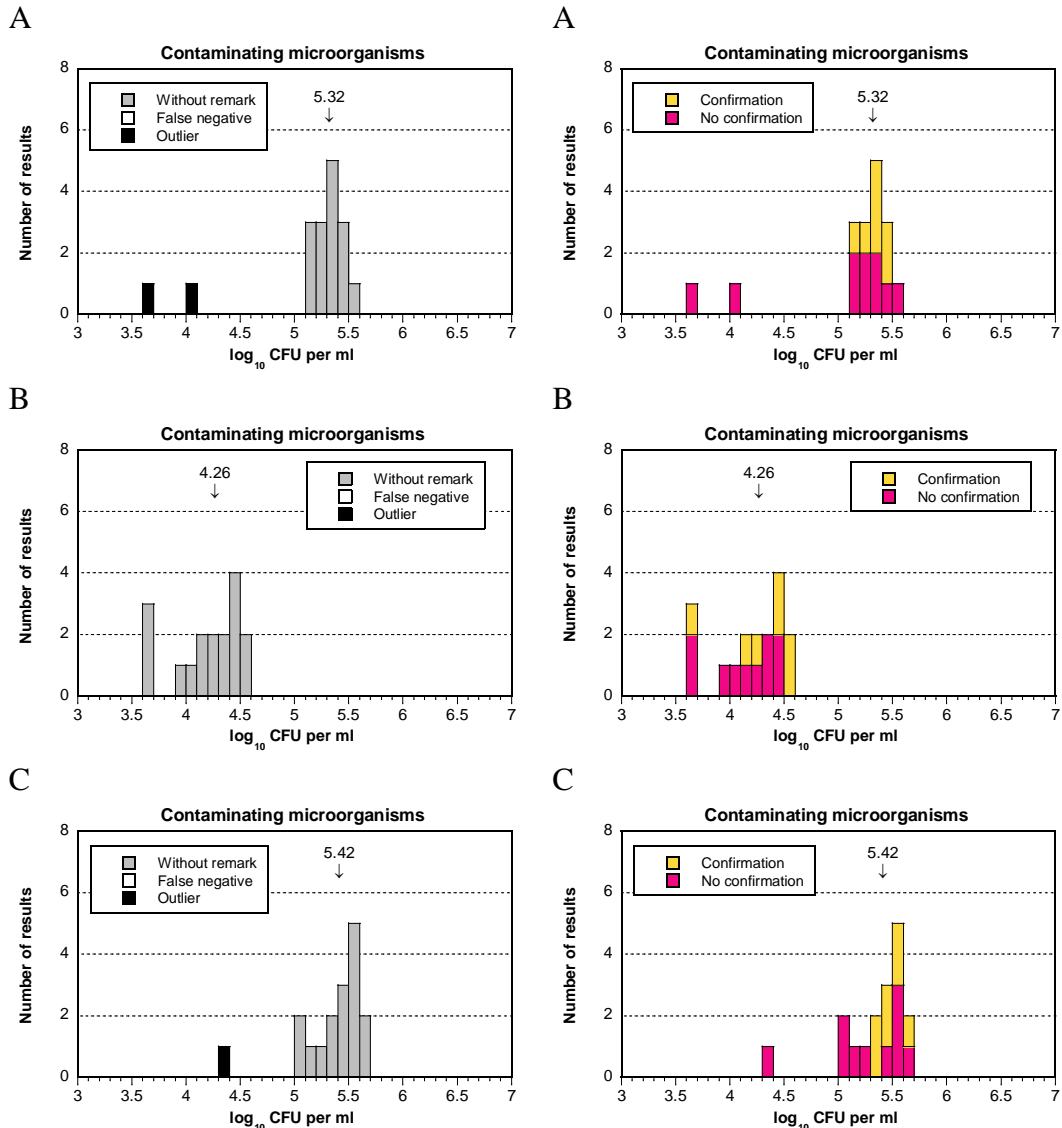
Two laboratories reported low results for mixture A, and one laboratory for mixture C. These results are difficult to explain – the strains present in the highest concentrations in these mixtures were catalase positive when tested at the National Food Agency. One possible explanation for the low results could be that ISO 13559:2002 / IDF 153:2002 stipulates the exclusion of pin-point colonies when counting. At the National Food Agency however, no ambiguity in colony size was noted during the counting, and all colonies in both mixture A and C were included without remark.

Results from 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	17	5.32	-	0	-	-	17	4.26	-	0	-	-	17	5.42	-	0	-	-
Confirmation	7	7	5.34	-	0	-	-	7	4.41	-	0	-	-	7	5.42	-	0	-	-
No confirmation*	10	10	5.22	-	0	-	-	10	4.20	-	0	-	-	10	5.37	-	0	-	-

Med: Median

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



Enterobacteriaceae

Mixture A

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

Mixture B

Strains of *Enterobacter aerogenes* and *Proteus mirabilis* were target organism for the analysis. At the National Food Agency, both small and large colonies were observed on violet red bile glucose agar (VRBG). Both colony types were oxidase negative, and therefore counted as Enterobacteriaceae. The results from the 145 laboratories were distributed well, with only two false negatives results and one high outlier.

Mixture C

A strain of *Escherichia coli* was target organism for the analysis in mixture C. At the National Food Agency, this formed distinct colonies on VRBG that were oxidase

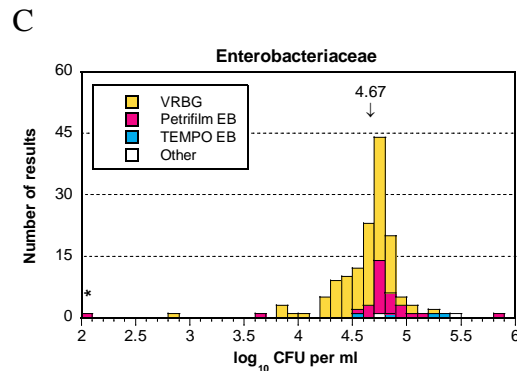
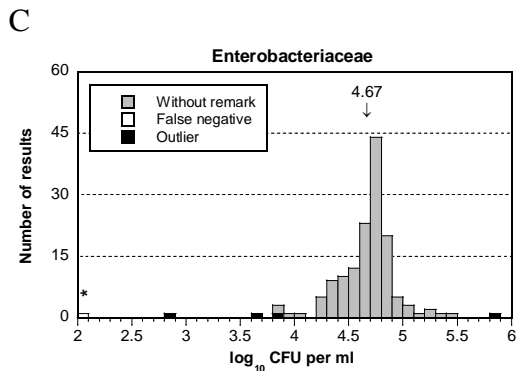
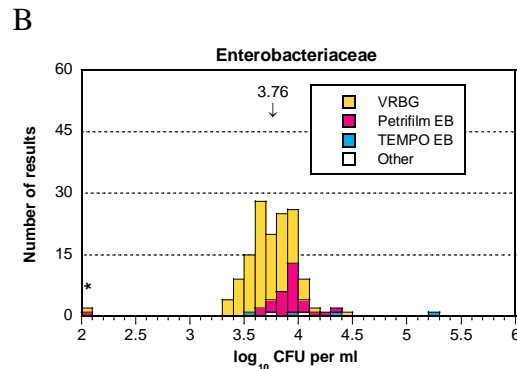
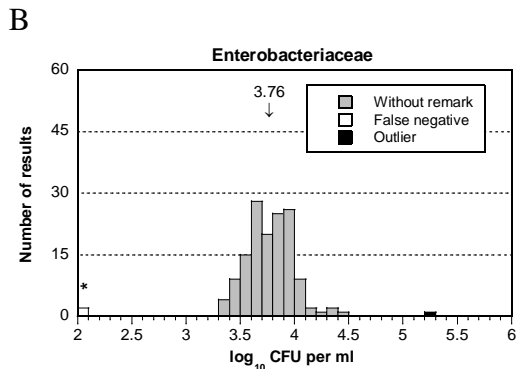
negative in subsequent confirmation tests. The results from the 145 laboratories that reported results were well distributed, with a distinct peak. The exceptions were a few outliers and one false negative result.

General remarks

As a whole, the analyses were unproblematic for the laboratories. The small number of false results and outliers could not be attributed to a specific standard, media or method for confirmation. As in previous proficiency testing rounds, the majority of the laboratories reported following either NMKL 144:2005 or ISO 21528-2:2004, with equivalent results. Most laboratories (76 %) used VRBG as media, while the majority of the remaining laboratories (20 %) used 3M™ Petrifilm™ Enterobacteriaceae (Petrifilm EB). For the latter media, there was a tendency to report higher results compared to VRBG, for both mixture B and C. It is possible that the strains in these mixtures grow better on Petrifilm EB than on VRBG, or that the colour indicator in Petrifilm EB assists in the counting of colonies. As in the analysis of aerobic microorganisms, slightly higher results were reported by those laboratories that used the fluorescence-based TEMPO® EB; a method that was however only used by 4 laboratories.

Results from analysis of Enterobacteriaceae

Media	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	143	141	-	-	2	-	-	142	3.76	0.21	2	0	1	140	4.67	0.25	1	3	1
VRBG	108	108	-	-	0	-	-	108	3.72	0.19	1	0	0	107	4.62	0.24	0	2	0
Petrifilm EB	29	28	-	-	1	-	-	29	3.92	0.15	1	0	0	27	4.79	0.12	1	1	1
TEMPO EB	4	4	-	-	0	-	-	3	-	-	0	0	1	4	-	-	0	0	0
Other	2	1	-	-	1	-	-	2	-	-	0	0	0	2	-	-	0	0	0



Coliform bacteria 30 °C and 37 °C

Mixture A

No target organism for coliform bacteria was present in mixture A. One false positive result was reported for each temperature.

Mixture B

A strain of *Enterobacter aerogenes* was target organism for the analysis and the majority of the reported results were without remark. However, a number of laboratories reported false negative results at 30 °C (6 of 57 laboratories) and 37 °C (8 of 100 laboratories). These false negative results were at both temperatures associated with the use of violet red bile agar (VRB). At the National Food Agency, two morphologically different colony types were observed on VRB. One was characteristic with a red zone of precipitation – the other consisted of small colonies with no precipitation. Only the characteristic colonies produced gas as a result of lactose fermentation in the subsequent confirmation in brilliant green lactose bile broth (BGLB). The production of gas was weak however, and could be interpreted as both positive and negative when BGLB was used. Therefore, negative results in such a confirmation test should also be regarded as correct. At the same time, problems with confirmation do not appear to have been an issue for the laboratories that used Petrifilm™ EC/CC and Petrifilm™ CC, where gas produced by lactose-fermenting coliform bacteria is trapped under a plastic film. Only 1 false positive result was reported for these two media.

As a consequence of the properties of the *E. aerogenes* strain, and the variation in results depending on what media was used, the results have not been evaluated and no z-scores have been calculated. The results are also excluded from the tables located below the box plots.

Mixture C

A strain of *Escherichia coli* was target organism for both analyses. The results from the analyses at 30 °C were well distributed and without remark, with the exception of 2 false negative results. Likewise at 37 °C the results were distributed well, though a few high and low outliers were reported, as were 3 false negative results. None of the deviating results could be attributed to the use of a specific method or media. At 37 °C, users of TSA/VRB reported somewhat higher results compared to users of other media. This is likely a results of the preincubation in TSA, which is a recommended step in NMKL 44:2004 if stressed coliform bacteria are expected to be present in the sample.

General remarks

For the majority of the laboratories, the analyses and results were without remark. The problems that were experienced mainly concerned confirmation of *E. aerogenes* in mixture B. False negative results could arise for the strain of *E. aerogenes* when using VRB, which is the prescribed media in NMKL 44:2004 and ISO 4832:2006. It should also be mentioned that there is a difference in how confirmation is carried out in these two methods. Whereas NMKL 44:2004 states that all presumptive colonies on VRB should be further confirmed in BGLB, ISO 4832:2006 stipulates that only atypical colonies require further confirmation. That users of Petrifilm™ simultaneously do not appear to have experienced problems with confirming coliform bacteria in the mixture, possibly indicates that the strain of *E. aerogenes* grew poorly in BGLB.

Nine laboratories reported using LSB/BGLB. The results for this combination of media were somewhat scattered and low and high outliers were reported for both

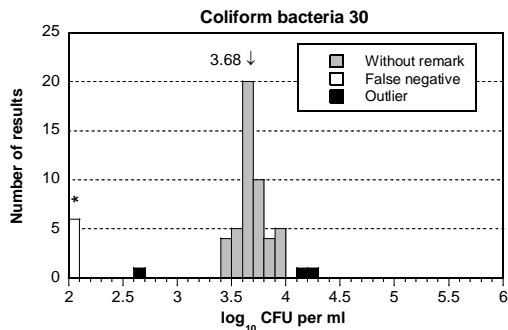
mixture B and C. Altogether, this resulted in mean values that deviated slightly from those from other media. LSB/BGLB was mainly used by laboratories that followed ISO 4831:2006 or NMKL 96 (different versions). ISO 4831:2006 is a method for detection of coliform bacteria that is based on MPN (Most Probable Number), and is intended to be used when the concentration of microorganisms is less than or equal to 100 CFU/g. NMKL 96 is likewise a MPN-based method, adapted for the analysis of coliform bacteria in fresh and frozen seafood, and intended to be used when the concentration of microorganisms is less than or equal to 300 CFU/g. Depending on what dilutions that were analysed, these two methods may possibly have been less reliable when analysing the high levels of microorganisms that were present in mixtures B and C.

Results from analysis of coliform bacteria, 30 °C

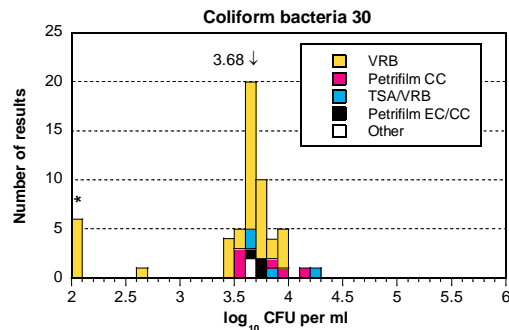
Media	N	Mixture A					Mixture B*					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	57	56	-	-	1	-	-	48	3.68	0.13	6	1	2	54	4.61	0.22	2	0	0
VRB	42	42	-	-	0	-	-	35	3.69	0.14	6	1	0	40	4.55	0.20	1	0	0
Petrifilm CC	6	5	-	-	1	-	-	5	3.67	0.19	0	0	1	5	4.81	0.06	1	0	0
TSA/VRB	4	4	-	-	0	-	-	3	-	-	0	0	1	4	-	-	0	0	0
Petrifilm EC/CC	3	3	-	-	0	-	-	3	-	-	0	0	0	3	-	-	0	0	0
Other	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	0	0

*The results for mixture B are not evaluated. A negative result may be considered acceptable, due to differences in methods and confirmation.

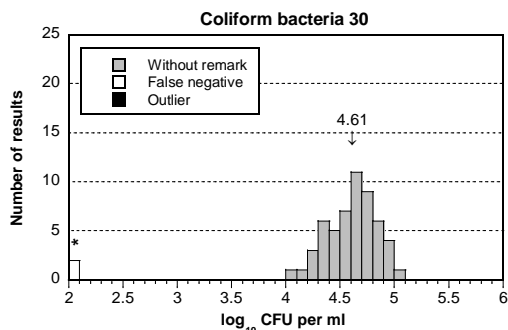
B



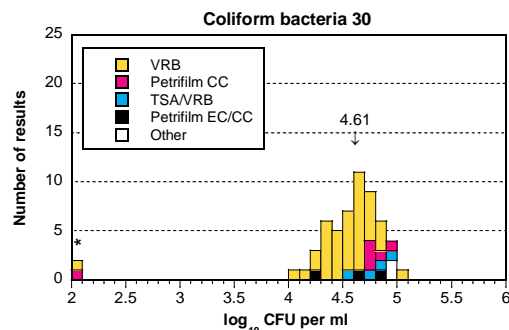
B



C



C

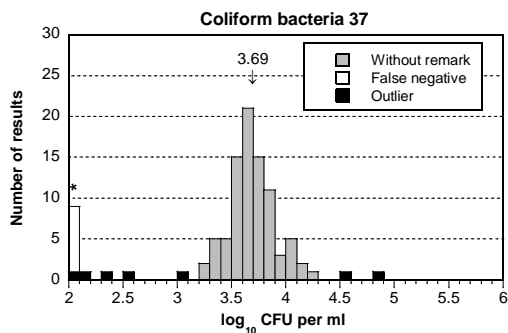


Results from analysis of coliform bacteria, 37 °C

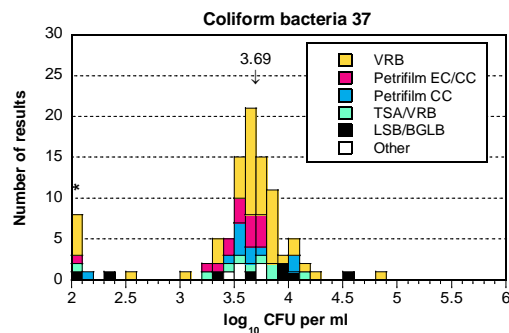
Media	N	Mixture A					Mixture B*					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	100	99	-	-	1	-	-	85	3.69	0.20	8	5	2	90	4.67	0.23	3	5	2
VRB	50	50	-	-	0	-	-	42	3.73	0.19	5	2	1	44	4.65	0.22	1	4	1
Petrifilm EC/CC	16	16	-	-	0	-	-	15	3.59	0.16	1	0	0	16	4.67	0.14	0	0	0
Petrifilm CC	11	10	-	-	1	-	-	10	3.68	0.22	0	1	0	10	4.68	0.21	1	0	0
TSA/VRB	9	9	-	-	0	-	-	8	3.68	0.26	1	0	0	9	4.90	0.14	0	0	0
LSB/BGLB	9	9	-	-	0	-	-	5	3.80	0.28	1	2	1	7	4.50	0.37	0	1	1
Other	5	5	-	-	0	-	-	5	3.60	0.13	0	0	0	4	-	-	1	0	0

*The results for mixture B are not evaluated. A negative result may be considered acceptable, due to differences in methods and confirmation.

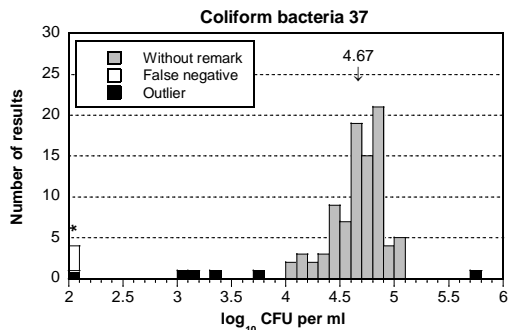
B



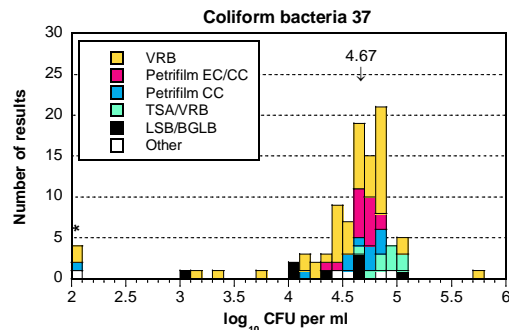
B



C



C



Thermotolerant coliform bacteria and Escherichia coli

Mixture A

No target organism for these analyses was present in mixture A. One laboratory reported a false positive result for thermotolerant coliform bacteria, and 3 laboratories reported false positive results for *E. coli*.

Mixture B

No target organism for these analyses was present in mixture B. Only 2 of 125 laboratories reported a false positive result for *E. coli*. In contrast, 10 of 51 laboratories reported a false positive result for thermotolerant coliform bacteria. The strain of *E. aerogenes* present in the mixture is not a thermotolerant coliform bacterium, but has in

an earlier proficiency testing round (October 2015) been shown form small colonies when incubated on violet red bile agar (VRB) at 43 °C. One explanation for false positive results could therefore be that the incubation temperature has been too low.

Mixture C

A strain of *Escherichia coli* was target organism for the analysis of thermotolerant coliform bacteria, and also for *E. coli*. The results for both analyses had a somewhat skewed distribution, with an over-representation of results lower than the main peak. A small number of low and high outliers were reported for both analyses, as where two false negative results.

General remarks

NMKL 125:2005 describes the analysis of both thermotolerant coliform bacteria and *E. coli*. The method defines thermotolerant coliform bacteria as those that form typical dark red colonies surrounded by a red zone of precipitation on VRB after a 24 h incubation at 44 °C. Confirmation is carried out by inoculating presumptive colonies into either *E. coli* broth (EC) or lactose tryptone lauryl sulphate broth (LTL SB). In these two media, thermotolerant coliform bacteria produce gas as a result of lactose fermentation. *E. coli* is further defined as those thermotolerant coliform bacteria that also produce indole, either in LTL SB or in tryptone broth. 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. On TBX, detection of *E. coli* is based on interaction between β -glucuronidase present in *E. coli* and an indicator in the media, which results in blue colonies. Further confirmation of β -glucuronidase positive colonies is not performed in ISO 16649-2:2001. 3M™ Petrifilm™ EC/CC and 3M™ Petrifilm™ SEC also detects *E. coli* based on the β -glucuronidase activity – these media however also detect production of gas resulting from fermentation of lactose.

For the analysis of thermotolerant coliform bacteria in mixture C, no clear difference could be seen between results from the different methods and media that were used. TSA/VRB was the main media used (25 of 51 laboratories), and did have a mean value that deviated slightly from that of other media. The remaining media were however all used by a small number (5-7) of laboratories, and the results from these were more scattered than those from VRB, which altogether makes it difficult to evaluate differences between the different media.

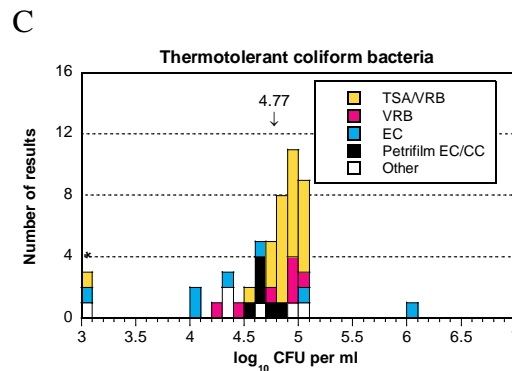
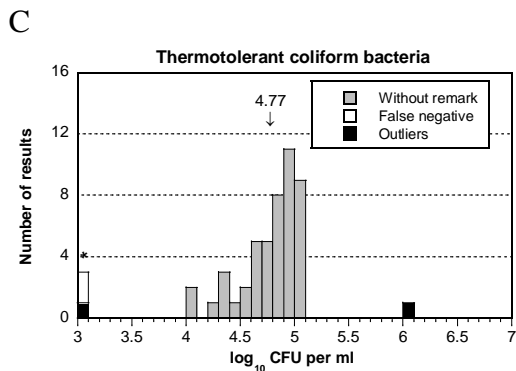
For the analysis of *E. coli* in mixture C, the results for TSA/VRB were somewhat higher than the mean value. In the same mixture, low results were associated with the use of TBX and 16649-2:2001. A weak β -glucuronidase activity can likely be ruled out as a cause of the low results for TBX, as users of Petrifilm EC/CC and Petrifilm SEC did not experience problems in identifying the strain of *E. coli*. This was true regardless of whether the incubation on Petrifilm was carried out at 37 °C or 44 °C. Low results for TBX have been seen in earlier proficiency testing rounds. Thus far, no unambiguous explanation for this has been identified. A contributing factor could however be if a preincubation step is carried out. When expecting the presence of stressed microorganisms in the sample, ISO 16649-2:2001 stipulates a preincubation at 37 °C for 4 h, prior to the final incubation at 44 °C for 18-24 h. As a comparison, in NMKL 125:2005 a preincubation is routinely carried out (1-2 h on TSA at 20-25 °C) prior to the final incubation on VRB. The use of TSA/VRB was also specified by the majority of the laboratories that followed NMKL 125:2005. When reporting results, both VRB and TSA/VRB are available as options for media. No corresponding option is however

available for TBX+preincubation, and therefore this needs to be specified manually by the participating laboratories when reporting the methods data. None of the laboratories that used TBX in this PT round reported performing a preincubation step, this however does not necessarily mean such a step was not carried out.

For the analysis of *E. coli*, a number of laboratories provided unclear information on their use of method and/or media. At the same time, a rather large number of methods/media were used by a mere 1-2 laboratories for this analysis, and consequently the group of Other/Unknown is quite large.

Results from analysis of thermotolerant coliform bacteria

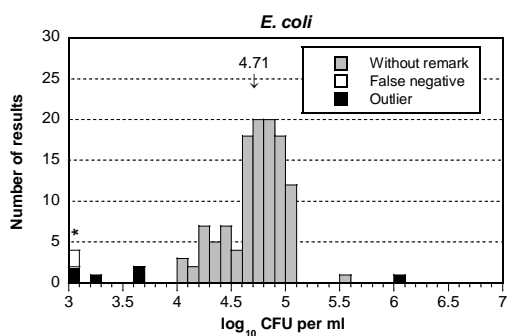
Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	51	50	-	-	1	-	-	41	-	-	10	-	-	47	4.77	0.26	2	1	1
TSA/VRB	25	24	-	-	1	-	-	22	-	-	3	-	-	24	4.89	0.12	1	0	0
VRB	7	7	-	-	0	-	-	6	-	-	1	-	-	7	4.76	0.29	0	0	0
EC	7	7	-	-	0	-	-	6	-	-	1	-	-	5	4.43	0.43	0	1	1
Petrifilm EC/CC	6	6	-	-	0	-	-	2	-	-	4	-	-	6	4.69	0.12	0	0	0
Other	6	6	-	-	0	-	-	5	-	-	1	-	-	5	4.67	0.31	1	0	0



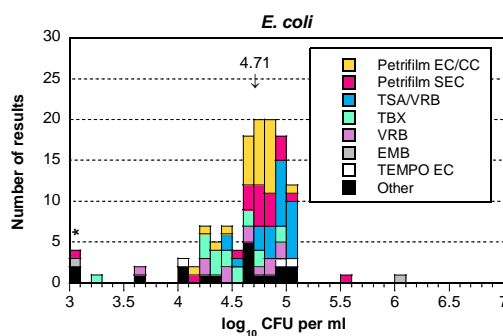
Results from analysis of Escherichia coli

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	125	122	-	-	3	-	-	123	-	-	2	-	-	117	4.71	0.26	2	5	1
Petrifilm EC/CC	28	28	-	-	0	-	-	27	-	-	1	-	-	28	4.70	0.19	0	0	0
Petrifilm SEC	19	17	-	-	2	-	-	19	-	-	0	-	-	19	4.80	0.26	1	0	0
TSA/VRB	25	25	-	-	0	-	-	25	-	-	0	-	-	25	4.87	0.17	0	0	0
TBX	17	17	-	-	0	-	-	17	-	-	0	-	-	16	4.54	0.24	0	1	0
VRB	12	11	-	-	1	-	-	11	-	-	1	-	-	11	4.64	0.26	0	1	0
EMB	3	3	-	-	0	-	-	3	-	-	0	-	-	0	-	-	0	1	1
TEMPO EC	3	3	-	-	0	-	-	3	-	-	0	-	-	3	-	-	0	0	0
Other	18	18	-	-	0	-	-	18	-	-	0	-	-	15	4.64	0.33	1	2	0

C



C



Presumptive *Bacillus cereus*

Mixture A

A strain of *B. cereus* was target organism for the analysis. The results were distributed well, and only 3 false negative results and 2 outliers were reported by the 122 laboratories that performed the analysis.

Mixture B

No target organism for the analysis was present in mixture B. Five of 119 laboratories reported a false positive result.

Mixture C

No target organism for the analysis was present in mixture C. Three of 119 laboratories reported a false positive result.

General remarks

Most laboratories followed either NMKL 67:2010 (59 %) or ISO 7932:2004 (22 %). Three laboratories reported following older versions of the NMKL method – NMKL 67:2003 or NMKL 67:1997. All three NMKL methods are based on incubation on blood agar (BA), but whereas the older methods prescribe confirmation of presumptive colonies on *Bacillus cereus* selective agar with Polymyxin (BcsA-P), NMKL 67:2010 allows confirmation to be carried out on either BcsA-P or Cereus-Ident-Agar (a chromogenic media). On BA, *B. cereus* forms large, irregular, greyish-white colonies that are surrounded by a well-defined zone of haemolysis. During confirmation on BcsA-P, presumptive *B. cereus* colonies are bluish, and surrounded by a zone of precipitation that is the result of lecithinase activity on egg yolk present in the media. On Cereus-Ident-Agar, presumptive *B. cereus* are blue-turquoise, and colonies are occasionally surrounded by a blue halo. ISO 7932:2004 prescribes inoculation on mannitol egg yolk Polymyxin agar (MYP), where presumptive *B. cereus* form large, pink colonies. These are normally surrounded by a zone of precipitation; again as a consequence of lecithinase activity. Confirmation in ISO 7932:2004 consists of streaking presumptive colonies onto BA; the presence of a zone of haemolysis is considered a positive result.

The reporting of method data for the analysis of presumptive *B. cereus* was unclear or ambiguous for several laboratories, which made it difficult to compare results from different methods and media. A number of laboratories stated they used methods and media that are not compatible, whereas others specified they used the same media in both steps in the analysis. Several laboratories reported that confirmation was

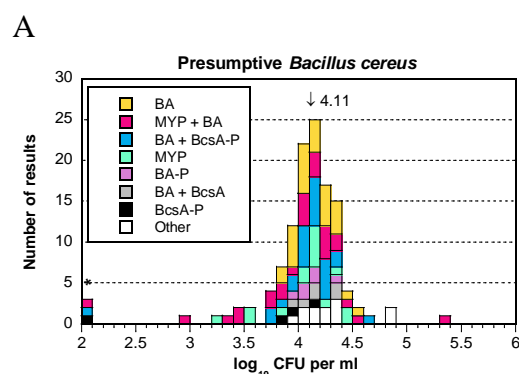
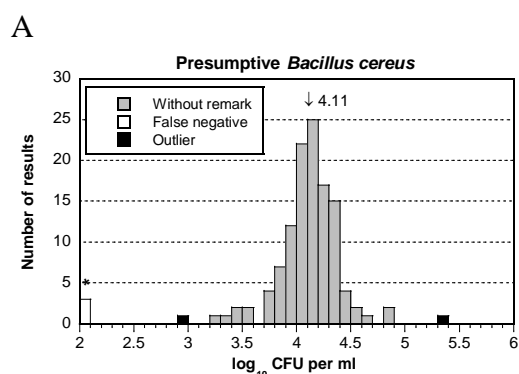
performed, but did not specify which media was used. Other laboratories stated they used “chromogenic” media, but without specifying this further. As a result, tables and figures below are based on the methods and media as they were reported by the laboratories, regardless if these are compatible or not. Laboratories that stated “chromogenic media” was used for the entire analysis are included in the group of “Other/unknown”. In cases where no media was provided for the confirmation step, it has been assumed that the laboratory used the media specified by the method they followed. Whether the discrepancies in the reporting of method data are a true reflection of how the analyses were carried out at the laboratories is difficult to determine.

Despite the variations in the reporting of methods data, the mean values for the various method groups are highly similar to each other. The distribution of results within the respective method groups is also quite narrow, with the exception of “MYP” and “MYP + BA”. Similar results were likewise reported regardless if NMKL 67:2010 or ISO 7932:2004 was used.

Results from analysis of presumptive *B. cereus*

Media	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	122	117	4.11	0.25	3	1	1	114	-	-	5	-	-	116	-	-	3	-	-
BA	28	28	4.13	0.18	0	0	0	27	-	-	1	-	-	28	-	-	0	-	-
MYP-BA	26	23	4.04	0.31	1	1	1	24	-	-	1	-	-	24	-	-	1	-	-
BA-BcsA-P*	25	24	4.10	0.20	1	0	0	21	-	-	3	-	-	23	-	-	1	-	-
MYP	15	15	4.04	0.35	0	0	0	15	-	-	0	-	-	15	-	-	0	-	-
BA-P*	6	6	4.10	0.11	0	0	0	5	-	-	0	-	-	5	-	-	0	-	-
BA-BcsA	6	6	4.17	0.18	0	0	0	6	-	-	0	-	-	6	-	-	0	-	-
BcsA-P*	4	3	-	-	1	0	0	4	-	-	0	-	-	4	-	-	0	-	-
Other	12	12	4.27	0.28	0	0	0	12	-	-	0	-	-	11	-	-	1	-	-

* P = addition of Polymyxin B (selective against Gram negative bacteria)



Coagulase-positive staphylococci

Mixture A

No target organism for this analysis was present in mixture A. At the National Food Agency, *Staphylococcus xylosus* formed characteristic grey, but coagulase negative colonies on Baird-Parker agar with rabbit plasma fibrinogen (BP + RPF), which may

have contributed to the reporting of false positive results by 14 of 120 laboratories. These false positive results were in 4 cases reported by the 19 users of 3M™ Petrifilm™ Staph Express, of which only 1 performed a subsequent confirmation test. The remaining 10 laboratories that reported false positive results used BP (in one case with the addition of RPF) and stated that confirmation was performed using latex agglutination test (4 laboratories), tube coagulase test (2 laboratories), Dry spot test (2 laboratories), VITEK® (1 laboratory), or did not state the method for confirmation (1 laboratory). Simultaneously, correct negative results were reported by other users of all these methods, which makes it difficult to find an explanation for the false positive results.

Mixture B

No target organism for this analysis was present in mixture B. Only 2 of 117 laboratories reported a false positive result.

Mixture C

A strain of *Staphylococcus aureus* was target organism for the analysis. The majority of the 120 reported results were distributed well, with a distinct peak. Still, 3 false negative results, as well as 1 high and 9 low outliers were reported. The methods used when reporting these false negative results and outliers was similar to mixture A; 4 laboratories used 3M™ Petrifilm™ and no confirmation, and 9 laboratories used BP and varying methods for confirmation.

General remarks

Most laboratories (45 %) followed NMKL 66:2009. Other methods used were ISO 6888-1:1999 (19 %), 3M™ Petrifilm™ Staph Express (16 %) and ISO 6888-2:1999 (9 %). The remaining 13 laboratories (11 %) either used odd methods (used by 2 laboratories or less) or did not state a method. Regardless of the choice of method and media, equivalent results were reported by the laboratories.

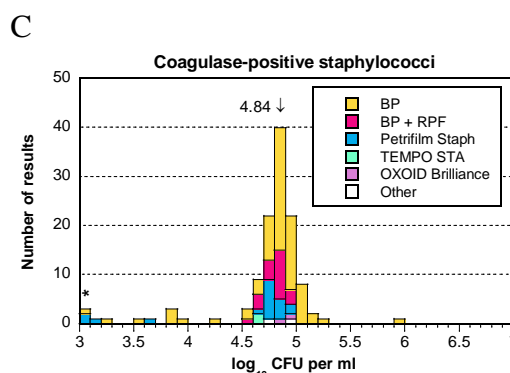
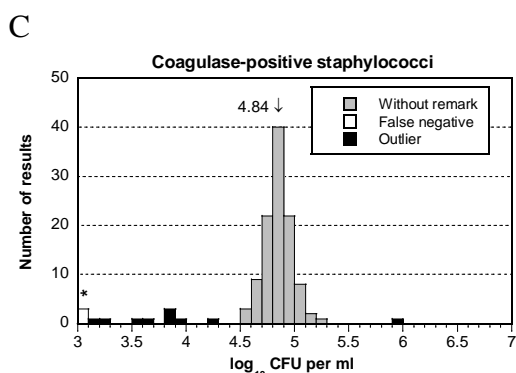
NMKL 66:2009 prescribes incubation on Baird-Parker (BP) and/or BP with rabbit plasma fibrinogen (BP + RPF). Blood agar (BA) can be used as a supplementary culture medium in addition to BP and BP + RPF. On BP, *S. aureus* forms characteristic shiny convex colonies that have a grey/black colour due to reduction of tellurite in the media. These are normally surrounded by a clear zone, due to lecithinase breakdown of egg yolk present in the media. An opaque ring may also form around the colony, as a result of lipolytic activity. Positive result in a subsequent coagulase test is used as confirmation. When using BP + RPF, the coagulase activity is tested directly on the agar plate, and no further confirmation is required according to the standard. Similar to NMKL 66, ISO 6888-1 stipulates the use of BP followed by confirmation with coagulase test, whereas ISO 6888-2 specifies the use of BP + RPF. The media in 3M™ Petrifilm™ Staph Express (Petrifilm Staph) is a modified Baird-Parker agar, with a chromogenic indicator that gives colonies of *S. aureus* a red/purple colour.

Traditionally, confirmation of coagulase-positive staphylococci is based on detection of extracellular or bound coagulase (tube coagulase test and slide coagulase test respectively). Here, several laboratories instead performed confirmation by a latex agglutination test, which is based on latex particles coated either with fibrinogen or with IgG, which 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 can also be carried out with a DNase test, something which is done with 3M™ Petrifilm™ Staph Express Disk. This test distinguishes microorganisms that

produce extracellular DNase (including *S. aureus*). False results and outliers were in this proficiency testing round reported by all of these methods for confirmation.

Results from analysis of coagulase-positive staphylococci

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	120	106	-	-	14	-	-	115	-	-	2	-	-	107	4.84	0.13	3	9	1
BP	74	65	-	-	9	-	-	70	-	-	1	-	-	65	4.87	0.13	1	7	1
BP + RPF	21	20	-	-	1	-	-	21	-	-	0	-	-	21	4.80	0.11	0	0	0
Petrifilm Staph	19	15	-	-	4	-	-	18	-	-	1	-	-	15	4.78	0.08	2	2	0
TEMPO® STA	2	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	0	0
OXOID Brilliance Staph 24	2	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	0	0
Other	2	2	-	-	0	-	-	2	-	-	0	-	-	2	-	-	0	0	0



Enterococci

Mixture A

Despite the fact that no target organism for this analysis was present in the mixture, 32 of 76 (42 %) laboratories reported a false positive result. At the National Food Agency, the strain of *P. acidilactici* formed atypical, slightly pink colonies on Slanetz & Bartley *Enterococcus*-agar (ENT). Upon confirmation on bile aesculin agar (BAA), these did not cause any blackening of the media after 2 hours, though a faint blackening could be seen after 24 hours. The high number of false positive results could not be attributed to a specific method or media. Possibly, the laboratories have different interpretations on how strong the blackening should be in order for a colony to be considered positive. The Swedish/Norwegian version of NMKL 68:2011 states that positive colonies give a “blackening” of the medium, whereas the English text uses the slightly wider definition “tan to black” colour. It could also be that a higher or lesser emphasis is given to blackening that appears after the 2 and 24 hours specified in NMKL 68:2011.

Three laboratories followed the drinking water standard ISO 7899-2:2000 (Detection and enumeration of intestinal enterococci), which is based on membrane filtering followed by incubation on ENT. As in the NMKL method, confirmation is carried out on BAA, but the incubation at 44 °C only lasts 2 hours. This is not sufficient time for the strain of *P. acidilactici* to cause blackening of the media, and likely contributed to

the fact that none of these laboratories reported a false positive result. It could also be mentioned that in the PT round October 2003, the same strain of *P. acidilactici* was distinguished from *Enterococcus* as it, in contrast to *Enterococcus*, does not grow in brain heart infusion broth (BHI) with 6.5 % salt or in BHI with pH 9.6. False positive results were however reported also by those laboratories that followed the older standard NMKL 68:2004, which includes confirmation with those methods.

Due to the difficulties in interpreting the results for the strain of *P. acidilactici*, and since NMKL 68:2004 does not strictly define the degree of blackening required, positive results are also considered correct. The analysis has therefore not been evaluated and no z-scores have been calculated. The results are also excluded from the tables located below the box plots.

Mixture B

A strain of *Enterococcus durans* was target organism for the analysis. The results from the 75 laboratories that performed the analysis were distributed well, but 4 laboratories reported false negative results, and 3 reported low outliers. At the National Food Agency, *E. durans* was observed as both small and large colonies on ENT, and a slight variation in colour could also be seen. During the subsequent confirmation on BAA, both types of colonies caused faint blackening of the media after 2 hours, which darkened to a distinct blackening after 24 hours.

Mixture C

A strain of *Enterococcus faecium* was target organism for the analysis. The results from the 76 laboratories that performed the analysis had a fairly narrow distribution, and 1 false negative result and 6 outliers were reported.

General remarks

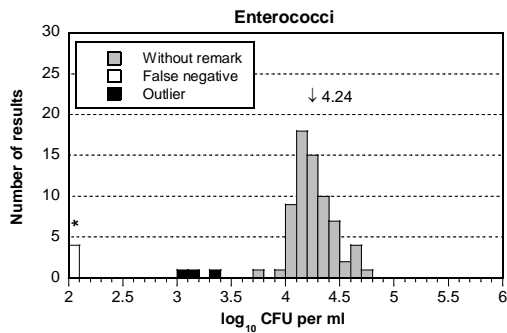
The majority of the laboratories (67 %) followed NMKL 68:2011. IDF 149A:1997 was used by 6 laboratories (8 %), with equivalent results as NMKL 68:2011 for all three mixtures. ENT was the most common media, and was in some cases preceded by a 2 hour preincubation on tryptone soya agar (TSA), as is recommended in NMKL 68:2011 if stressed bacteria are expected to be present in the sample. The remaining methods were all used by 3 or fewer laboratories, and are for that reason difficult to evaluate.

Results from analysis of enterococci

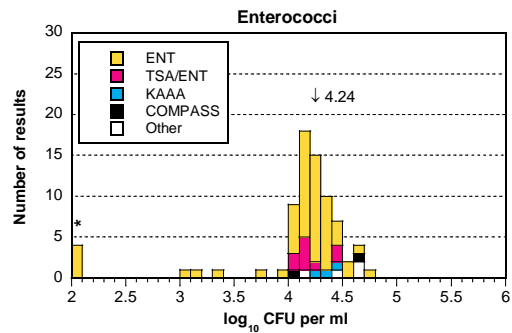
Media	N	Mixture A*						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	76	44	-	-	32	-	-	68	4.24	0.18	4	3	0	69	4.81	0.10	1	6	0
ENT	58	33	-	-	25	-	-	50	4.23	0.17	4	3	0	53	4.83	0.11	1	4	0
TSA/ENT	9	4	-	-	5	-	-	9	4.19	0.14	0	0	0	8	4.74	0.06	0	1	0
KAAA	3	1	-	-	2	-	-	3	-	-	0	0	0	3	-	-	0	0	0
COMPASS	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	0	0
Other	4	4	-	-	0	-	-	4	-	-	0	0	0	3	-	-	0	1	0

*The results for mixture A are not evaluated. Positive results could also be considered correct, depending on differences in methods and confirmation.

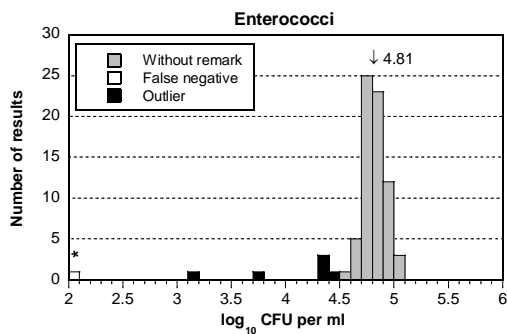
B



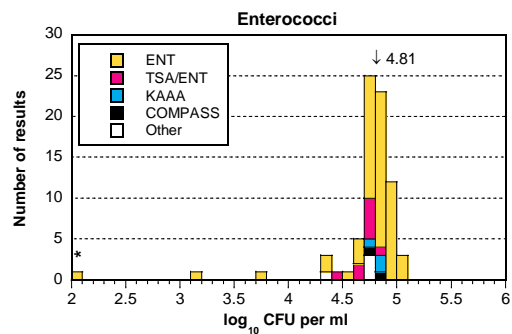
B



C



C



Gram-negative bacteria in dairy products

Mixture A

No target organism for this analysis was present in mixture A. Only one laboratory reported a false positive result.

Mixture B

Strains of *Enterobacter aerogenes* and *Proteus mirabilis* were target organisms for the analysis. All 12 laboratories that performed the analysis reported a correct result.

Mixture C

A strain of *Escherichia coli* was target organism for the analysis. All 12 laboratories that performed the analysis reported a correct result.

General remarks

Only 12 laboratories performed the analysis. All reported that violet red bile glucose agar (VRBG) was used as media, and 10 of 12 specified following NMKL 192:2011. The method described in NMKL 192:2011 detects recontamination of Gram-negative bacteria in milk and cream. These bacteria do not survive high temperature/short time (HTST) pasteurisation, where the temperature is increased to 72 °C for at least 15 seconds. Presence of Gram-negative bacteria therefore indicates contamination has taken place after the pasteurisation process, something that may limit the shelf life. The standard prescribes preincubation of the package of milk or cream at 25 °C / 24 h, or at room temperature for 28 h, followed by spreading of 10 µl and 100 µl, respectively, onto VRBG plates. Incubation is at 30 °C for 24 h. The method is qualitative, and the presence of 5 or more colonies is considered a positive result. Confirmation is done by

transferring colonies with a loop onto a glass slide with potassium hydroxide. Formation of a viscous string after 5-10 seconds of mixing is considered as a positive result for the presence of Gram-negative bacteria.

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	12	11	1	12	0	12	0
NMKL 192:2011	10	9	1	10	0	10	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.

When laboratories appear to have mistakenly analysed the wrong mixture, the corresponding results are written in italics. In this proficiency testing round, one laboratory (4352) appears to have mixed up samples A and C.

Z-scores for individual analyses are shown in Annex 2 (see below) 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

Z-scores, box plots and deviating results

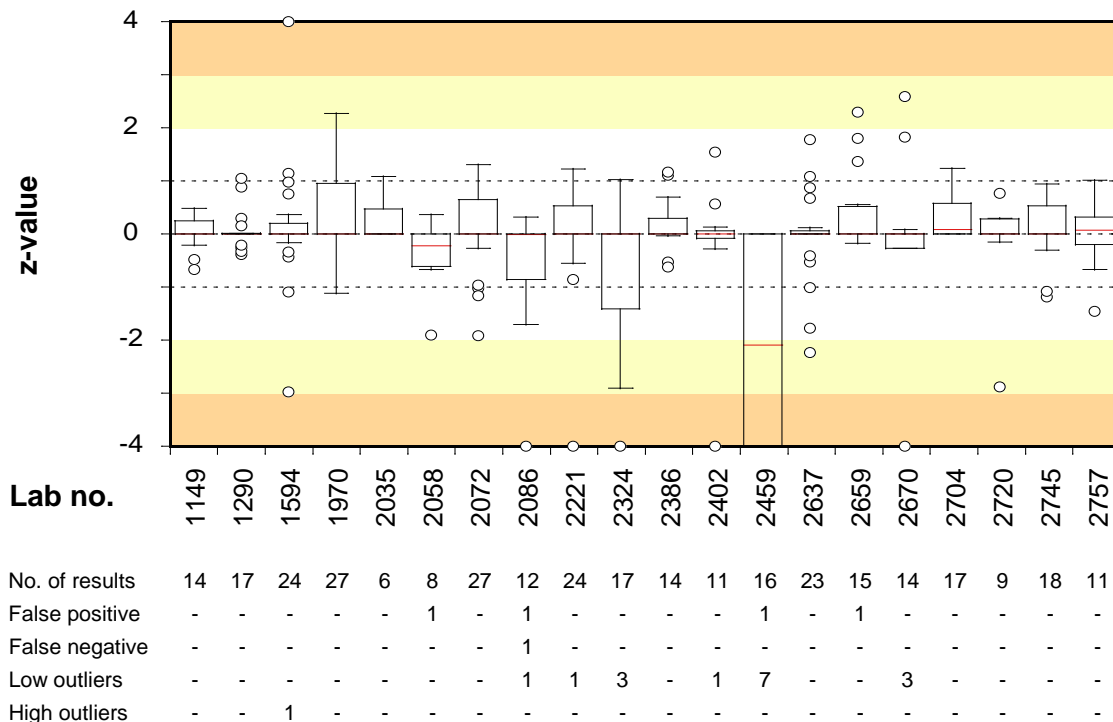
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

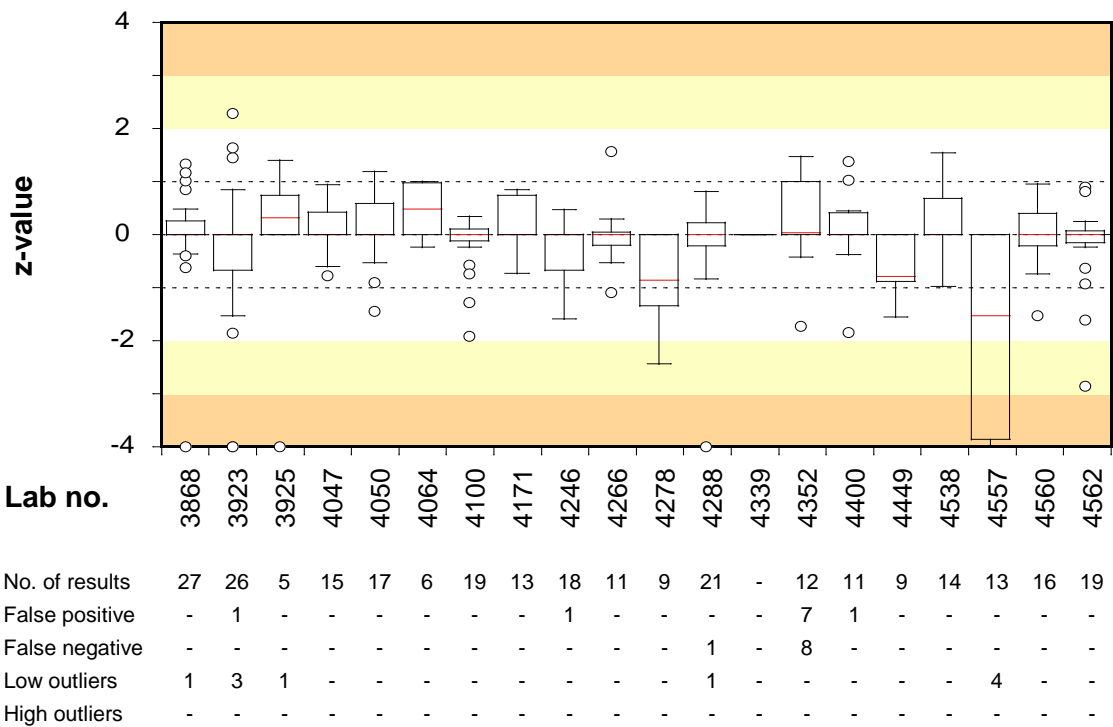
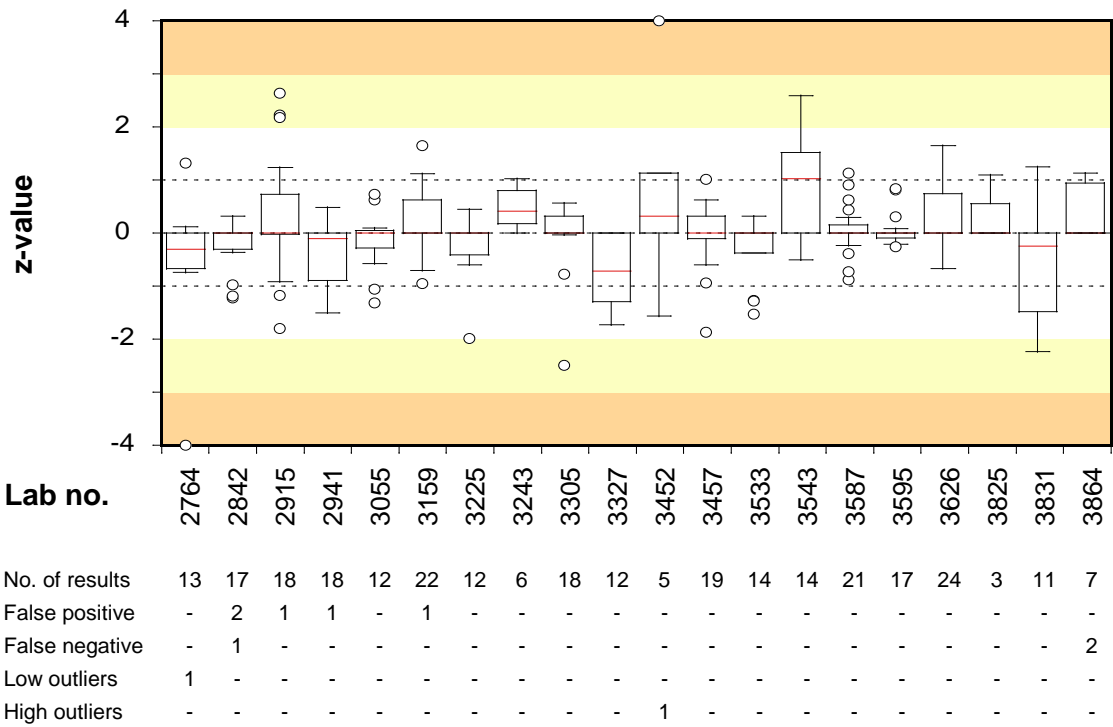
The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates the results of that individual laboratory, with false results excluded, are close to the general mean values calculated for all laboratory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

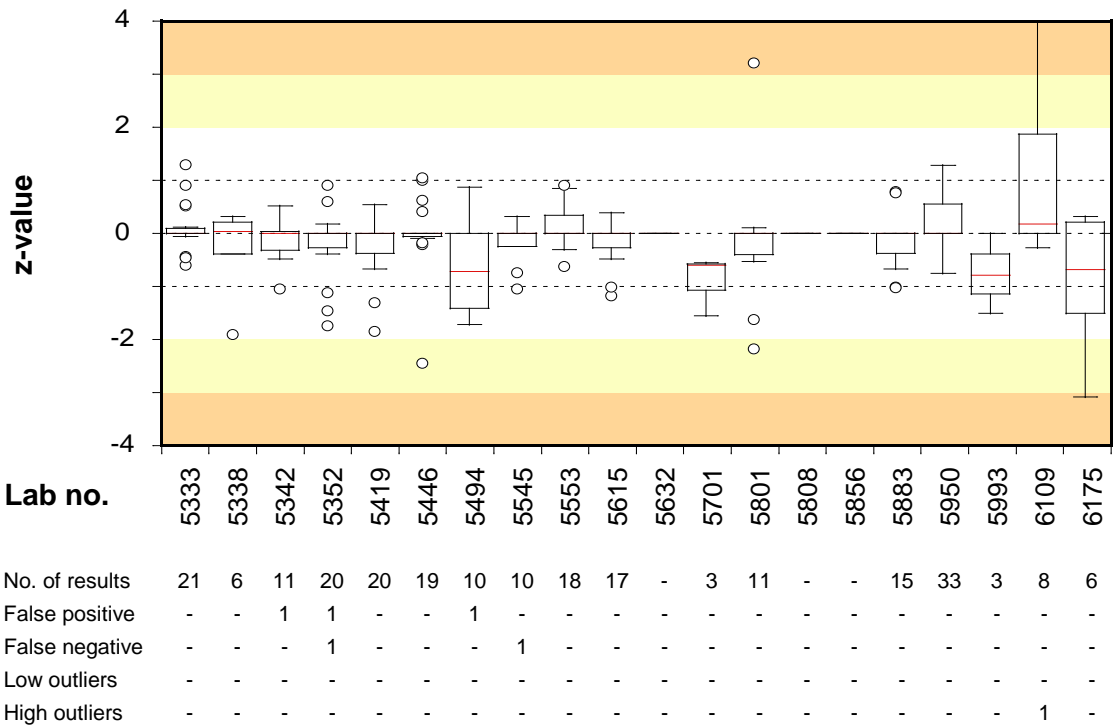
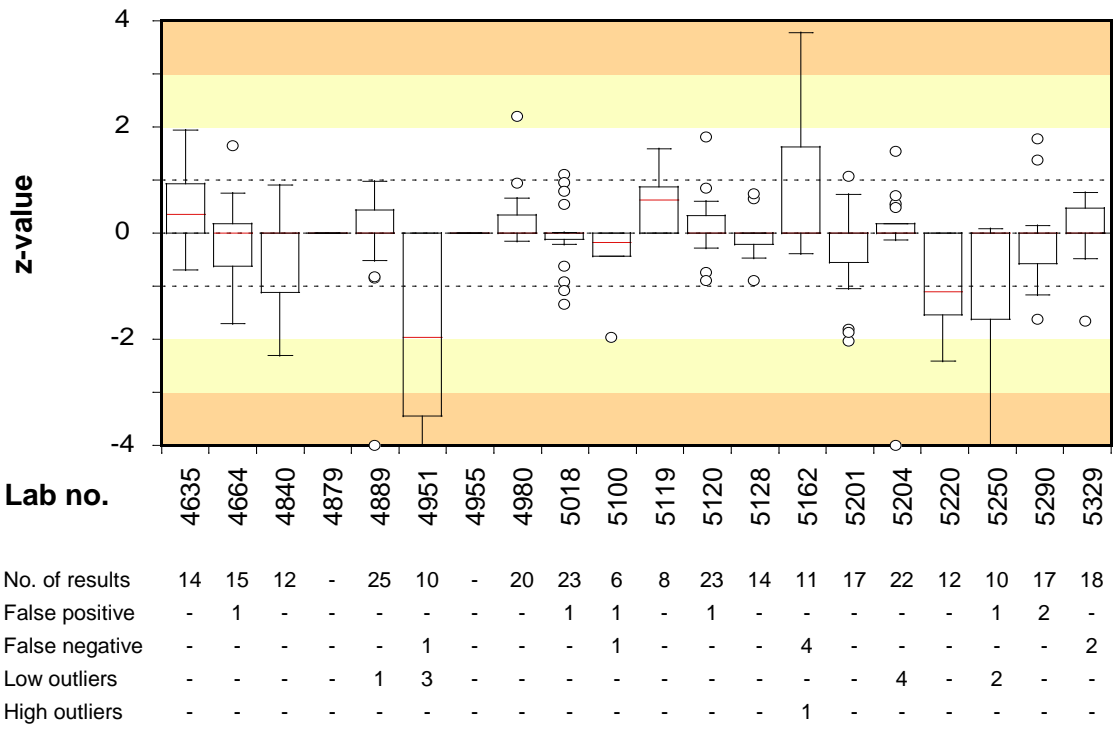
Box plots and numbers of deviating results for each laboratory

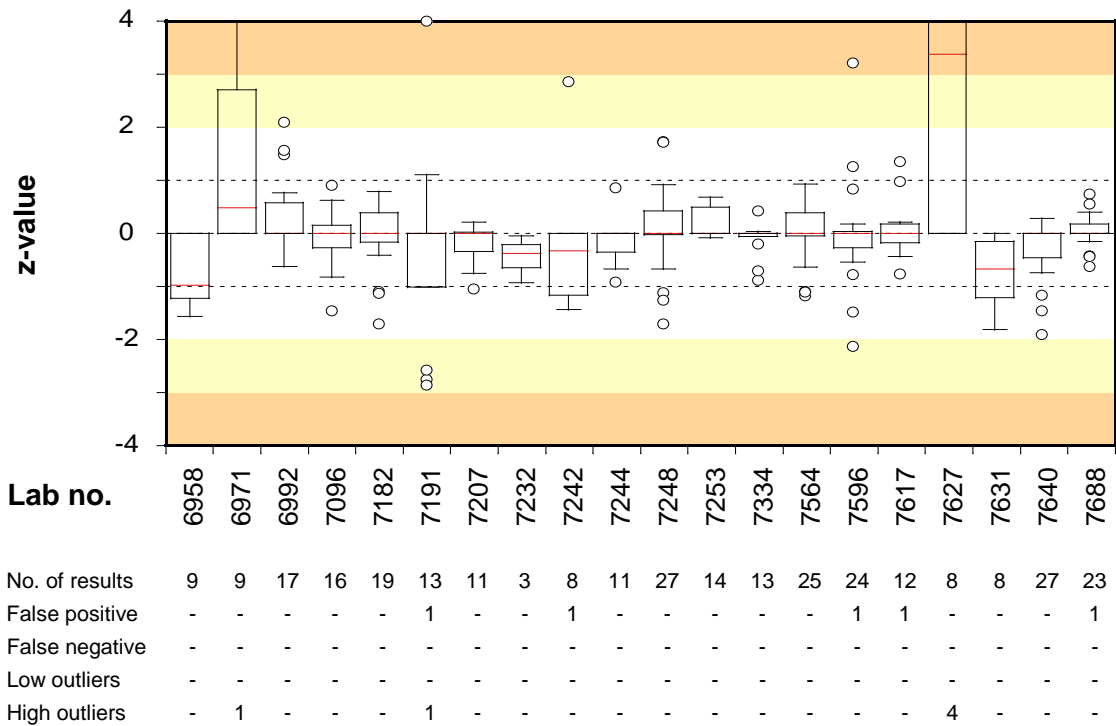
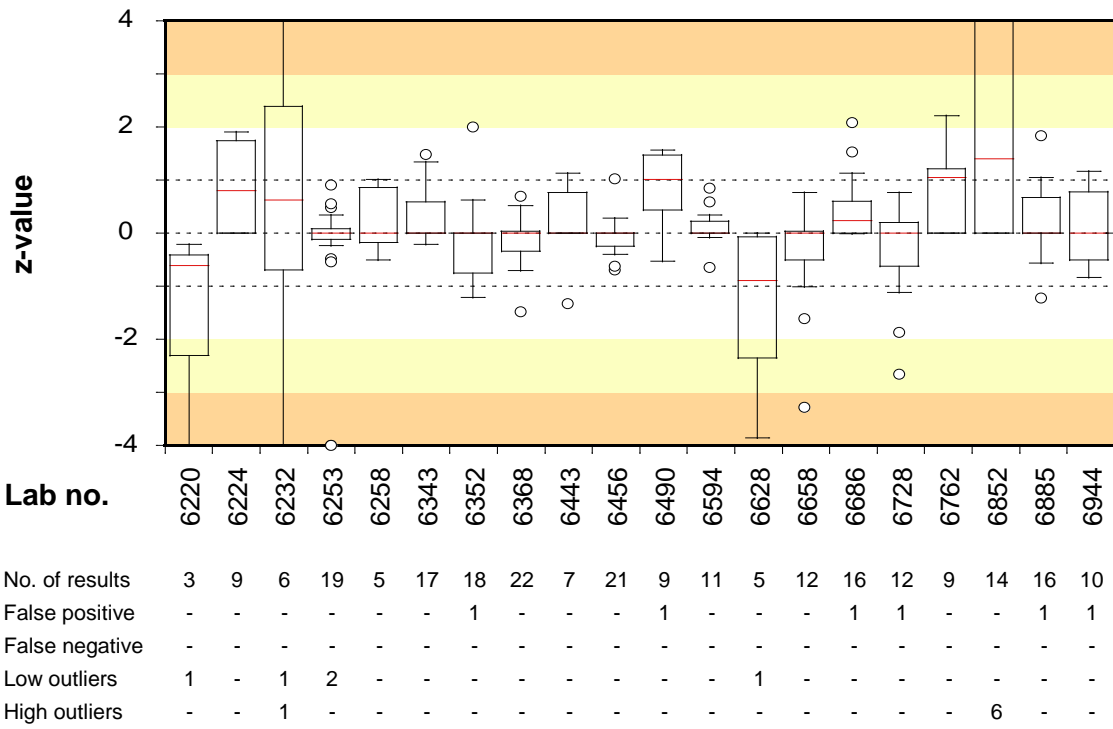
- Z-scores are calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories.
- Outliers are included in the figures after being 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-value 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 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 that the results are located in.

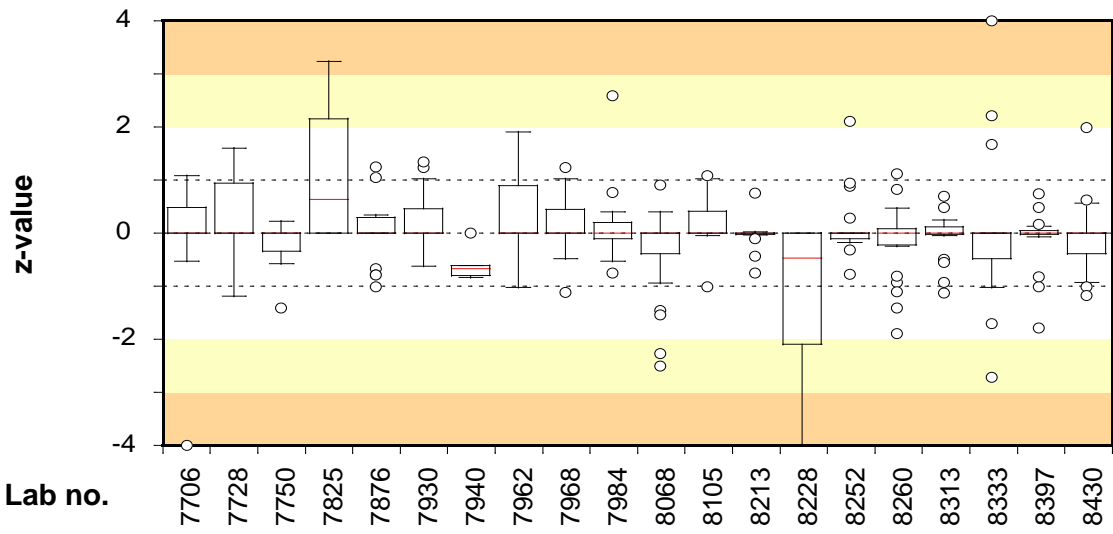
* $< [lowest\ result\ in\ the\ box - 1,5 \times (highest\ result\ in\ the\ box - lowest\ result\ in\ the\ box)]$
or
 $> [highest\ result\ in\ the\ box + 1,5 \times (highest\ result\ in\ the\ box - lowest\ result\ in\ the\ box)]$.



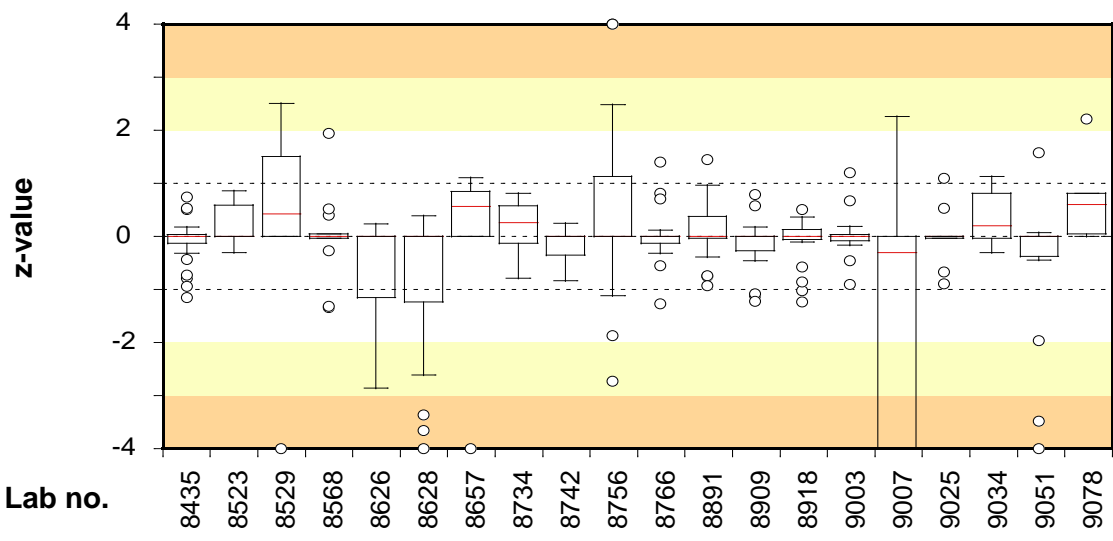




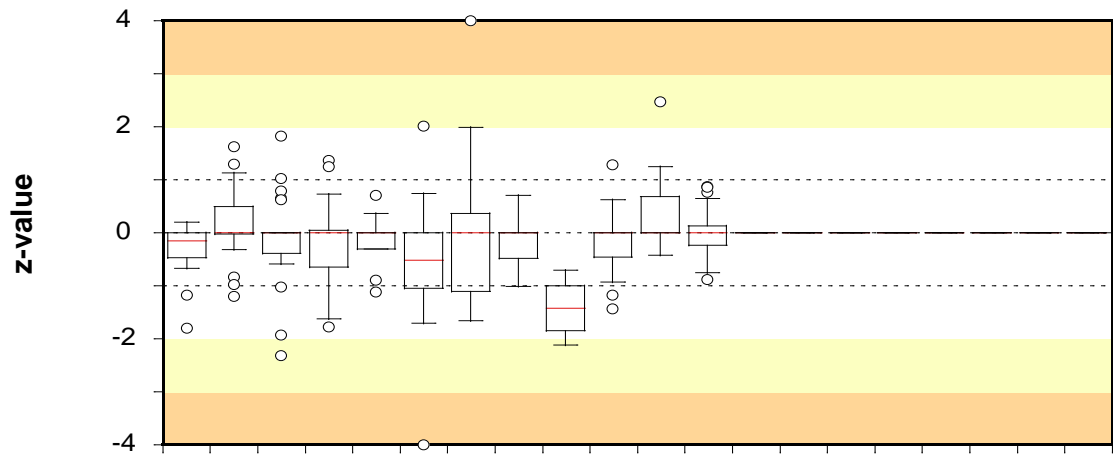




Lab no.	7706	7728	7750	7825	7876	7930	7940	7962	7968	7984	8068	8105	8213	8228	8252	8260	8313	8333	8397	8430
No. of results	17	19	11	16	17	23	5	24	24	12	27	11	15	14	17	24	19	13	17	14
False positive	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	2	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-



Lab no.	8435	8523	8529	8568	8626	8628	8657	8734	8742	8756	8766	8891	8909	8918	9003	9007	9025	9034	9051	9078
No. of results	27	9	20	13	17	27	6	6	20	17	17	20	19	20	16	11	9	12	15	6
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	1	-	-	2	-	-	-	-	-	-	-	-	-	5	-	-	1	-
High outliers	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-



Lab no.	9217	9429	9436	9453	9512	9559	9655	9662	9747	9763	9890	9903	9950
No. of results	11	24	24	17	9	18	16	19	4	18	20	23	-
False positive	-	-	-	-	-	1	-	2	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	1	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	1	-	-	-	-	-	-

Test material and quality control

Test material

Each laboratory received three freeze-dried microbial mixtures, designated A-C. The manufactured test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (3). Each laboratory received one vial of each mixture. 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	Mikroorganism	Strain	
		SLV no. ²	Reference ³
A	<i>Pediococcus acidilactici</i>	SLV-213	CCUG 45146
	<i>Staphylococcus xylosus</i>	SLV-283	Cheese
	<i>Bacillus cereus</i>	SLV-518	CCUG 44741
B	<i>Enterococcus durans</i>	SLV-078	CCUG 44816
	<i>Enterobacter aerogenes</i>	SLV-099	ATCC 13048
	<i>Proteus mirabilis</i>	SLV-180	CCUG 48088
C	<i>Staphylococcus saprophyticus</i>	SLV-013	CCUG 45100
	<i>Escherichia coli</i>	SLV-085	Water
	<i>Staphylococcus aureus</i>	SLV-280	Egg
	<i>Enterococcus faecium</i>	SLV-459	CCUG 35172

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 ²			C ¹		
	<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 PCA according to NMKL no. 86	5.332	1.19	1.49	4.431	1.50	5.73	5.539	1.39	4.80
Aerobic microorganisms, 20 °C PCA according to NMKL no. 86	5.330	1.15	1.01	4.932	1.29	2.17	5.522	1.17	0.97
Contaminating microorganisms SFA according to ISO no. 13559/ IDF no. 153:2002	5.341	1.12	0.65	4.391	1.27	1.94	5.541	1.22	1.76
Enterobacteriaceae VRGG according to NMKL no. 144	-	-	-	4.022	1.16	0.60	4.769	1.49	2.13
Coliform bacteria 30 °C VRB according to NMKL no. 44	-	-	-	3.601	1.21	0.38	4.704	1.39	1.43
Coliform bacteria 37 °C VRB according to NMKL no. 44	-	-	-	3.631	1.25	0.54	4.723	1.48	1.86
Thermotolerant coliform bacteria TSA/VRB according to NMKL no.125	-	-	-	-	-	-	4.953	1.34	2.21
<i>Escherichia coli</i> TSA/VRB according to NMKL no. 125	-	-	-	-	-	-	4.953	1.34	2.21
Presumptive <i>Bacillus cereus</i> BA according to NMKL no. 67	4.282	1.26	1.24	-	-	-	-	-	-
Coagulase-positive staphylococci BP+RPF according to NMKL no. 66	-	-	-	-	-	-	4.885	1.27	1.13
Enterococci ENT according to NMKL no. 68	4.238*	1.39*	2.36*	4.227	1.45	2.80	4.755	1.30	0.96
Gram-negative bacteria in pasteurised milk and cream. Detection of recontamination. VRBG according to NMKL no. 192	Neg.	-	-	Pos.	-	-	Pos.	-	-

- No target organism and therefore no value

¹ n = 10 vials analysed in duplicate

² n = 5 vials analysed in duplicate

* The values refer to the colonies of *P. acidilactici* that at the National Food Agency were considered false positive.

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58-64.
2. Anonym, 2012. Verksamhetsprotokoll. Mikrobiologi. Dricksvatten & Livsmedel, Livsmedelsverket.
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.

Lab no	Code no	Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorg. in milk products			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				
8397	3 1 2	0.740	-1.785	0.052							0	0.130	0.486																							8397		
8430	1 2 3	-0.159	1.994	-1.015							0	0.564	-1.176	0	-0.931																				8430			
8435	1 2 3	0.178	0.142	-0.215	-1.148	0.069	0.505				0	-0.786	-0.731	0	-0.435	0	-0.312	0	0	0.526	0	0	0.746	-0.027	0	0	0	0	-0.935	0.092	-0.045			8435				
8523	3 1 2	0.628	-0.303	0.585							0	0.468	-0.001																						8523			
8529	3 2 1	1.358	1.772	2.007							0	0.854	1.662							0	0	1.070	0	0	1.280	1.839	0	0	0	0	2.502	-4.000	1.322		8529			
8568	2 3 1	-0.271	-1.340	-0.037							0	-1.317	0.405							0		0.515				-0.027	0	0				1.943	0.052		8568			
8626	2 3 1	-0.103	-0.377	0.052	-1.148	0.235	-0.976				0	-1.848	0.040							0		-2.749	0	0	-2.853	0	0	-2.573							8626			
8628	2 1 3	-2.294	0.142	-2.614	-4.000	-0.427	-2.551				0	0.227	-3.366	0	0.197	0	0.385	0	0	-0.756	0	0	-0.513	-1.616	0	0	0	0	-0.857	-0.289	-3.660			8628				
8657	3 2 1	-4.000	1.105	0.852							0	0.564	0.567																							8657		
8734	2 3 1	0.572	0.808	-0.126							0	-0.786	0.527																							8734		
8742	2 1 3	-0.552	-0.822	-0.837							0	-0.256	0.243							0		-0.051	0	0	-0.445	0	0	-0.208	-0.663	0	0	0	0	-0.154		8742		
8756	2 1 3	1.133	4.000	-0.126							0	4.000	0.527																							8756		
8766	2 3 1	-0.552	0.808	-0.126							0	-1.269	0.121																							8766		
8891	1 2 3	0.965	-0.748	-0.392				1.449	0.703	0.513	0	-0.738	0.243	0	-0.931																					8891		
8909	2 1 3	0.178	-0.451	-0.037							0	0.130	-0.203	0	-1.111																					8909		
8918	1 3 2	-0.103	0.512	-0.570				-1.229	0.052	0.128	0	0.227	0.364							0		0.298				0	0	0.135	-1.020	0	0	0	0	-0.857		8918		
9003	1 2 3	-0.159	-0.103	-0.464							0	1.206	0.186	0	0.676	0	0.080																			9003		
9007	3 1 2	2.257	-4.000	-4.000							0	-0.304	-4.000																							9007		
9025	3 2 1	-0.665	-0.896	-0.037							0	1.095	0.527																							9025		
9034	3 1 2	1.133	0.808	0.763	0.805	0.979	0.413				0	-0.304	-0.285																							9034		
9051	1 3 2	1.583	0.068	-0.304							0	-0.449	-0.122																								9051	
9078	3 2 1	0.684	2.216	0.052							0	0.516	0.810																								9078	
9217	3 1 2	-0.271	-0.155	-0.037							0	-1.172	0.202																								9217	
9429	2 3 1	0.122	1.624	1.296				0.189	1.106	-0.311	0	0.999	-0.203	0	-1.202	0	-0.834																				9429	
9436	1 2 3	-0.103	-0.377	1.829							0	-1.028	-0.406	0	-1.924	0	-2.314	0	0	0.643	0	0	0.784	-0.583	0	0	0	0	0.627	-0.562	1.029				9436			
9453	2 3 1	-0.047	-0.377	-0.659				1.370	-1.776	0.732	0	-0.642	-1.622																								9453	
9512	2 3 1	-1.114	-0.896	-0.304							0	0.709	0.364																								9512	
9559	1 2 3	-0.440	-1.044	2.007	-0.634	-0.593	-1.162	-0.677	-0.537	-4.000	0	-1.703	-0.082																								9559	
9655	2 3 1	0.740	1.994	0.763							0	-0.738	-1.501	0	-1.472	0	-1.617																				9655	
9662	1 2 3	-0.665	-0.525	-1.015							0	0.227	0.081	0	-0.435	0	-0.355																				9662	
9747	3 1 2	-1.283	-1.563	-2.116							0																											9747
9763	1 3 2	-0.215	-0.451	-0.926							0	0.564	-1.176	0																							9763	
9890	3 1 2	1.077	1.253	0.052	0.908	2.469	-0.421				0	0.902	0.202																								9890	
9903	1 2 3	-0.665	-0.748	0.763	-0.326	-0.179	0.876				0	-0.883	-0.731																								9903	
9950	2 3 1																																					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 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