

Food Microbiology

April 2015



Edition

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Proficiency Testing
Microbiology – Food
April 2015



1457
ISO/IEC 17043

Quantitative analyses

- Aerobic microorganisms, 30 °C
- Psychrotrophs
- Enterobacteriaceae
- *Escherichia coli*
- Presumptive *Bacillus cereus*
- Coagulase positive staphylococci
- Lactic acid bacteria
- *Clostridium perfringens*
- Anaerobic sulphite reducing bacteria
- Aerobic microorganisms in fish products, 20-25 °C
- H₂S producing bacteria in fish products
- Yeasts
- Moulds

Abbreviations

Media

BA	Blood Agar
BcsA	Bacillus cereus selective Agar
BP	Baird-Parker agar
DG 18	Dichloran Glycerol agar
DRBC	Dichloran Rose Bengal Chloramphenicol agar
ISA	Iron Sulphite Agar
MPCA	Milk Plate Count agar
MPN	Most Probable Number
MRS	de Man, Rogosa and Sharpe agar
MRS-aB	de Man, Rogosa and Sharpe agar with amphotericin
MRS-S	de Man, Rogosa and Sharpe agar with sorbic acid
MYP	Mannitol egg Yolk Polymyxin agar / Mossel agar
OGYE	Oxytetracycline Glucose Yeast Extract agar
P	Polymyxin
PCA	Plate Count Agar
RPF	Rabbit Plasma Fibrinogen
SFP	Shahidi Ferguson Perfringens agar base
TBX	Tryptone Bile X-glucuronide agar
TGE	Tryptone Glucose Extract agar
TSA	Trypticase Soy Agar
TSC	Tryptose Sulphite Cycloserine agar
VRB	Violet Red Bile agar
VRBG	Violet Red Bile Glucose agar
YGC	Yeast extract Glucose Chloramphenicol agar

Organisations

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

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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. several laboratories state a medium that is not mentioned in the standard method. Therefore, in the following section, results have been grouped according to the method or the medium used to perform the analysis.

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 participants results.




Tables and figures legend

Tables

n	number of laboratory that performed the analysis
m	results mean value in \log_{10} cfu/ml (false results and outliers excluded)
s	results 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 all analytical results obtained 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

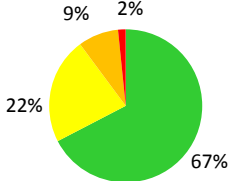
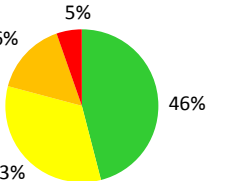
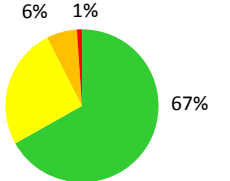
Results of the PT round April 2015

General outcome

Samples were sent to 188 laboratories, 45 in Sweden, 125 in other European countries, and 18 outside Europe. Results were reported from 187 laboratories; 135 (72 %) provided at least one result that received an annotation. In the previous round (April 2014) with similar analyses, the proportion was 81 %.

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 Mixtures content and % of deviating results (F%: false result, Out: outliers).

	Mixture A			Mixture B			Mixture C		
% participants with									
	9% 2% 22% 67%			16% 5% 33% 46%			6% 1% 26% 67%		
<ul style="list-style-type: none"> ■ 0 annotation ■ 1 annotation ■ 2 annotations ■ >2 annotations 									
Organisms	<i>Lactobacillus plantarum</i> <i>Escherichia coli</i> <i>Kluyveromyces marxianus</i> <i>Penicillium verrucosum</i>			<i>Aeromonas hydrophila</i> <i>Clostridium perfringens</i> <i>Staphylococcus warneri</i> <i>Staphylococcus aureus</i> <i>Shewanella putrefaciens</i>			<i>Micrococcus sp.</i> <i>Escherichia coli</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i>		
Analysis	Target	F%	Out	Target	F%	Out	Target	F%	Out
Aerob. microorg. 30 °C	<i>L. plantarum</i> <i>E. coli</i>	0	3	<i>A. hydrophila</i> <i>S. warneri</i> <i>S. aureus</i> <i>S. putrefaciens</i>	0	6	<i>Micrococcus</i> <i>S. aureus</i>	0	3
Psychrotrophs	<i>L. plantarum</i> <i>E. coli</i> <i>P. verrucosum</i>	46	0	<i>A. hydrophila</i> <i>S. warneri</i> <i>S. aureus</i> <i>S. putrefaciens</i>	47	0	<i>Micrococcus</i> <i>S. aureus</i>	0	0
Enterobacteriaceae	<i>E. coli</i>	1	5	(<i>A. hydrophila</i>)	43	-	<i>E. coli</i>	1	5
<i>E. coli</i>	<i>E. coli</i>	0	6	-	2	-	<i>E. coli</i>	3	8
Presump. <i>B. cereus</i>	-	1	-	(<i>A. hydrophila</i>)	14	-	<i>B. cereus</i>	2	1
Coagulase- positive Staphylococci	-	0	-	(<i>S. warneri</i>) <i>S. aureus</i>	7	14	<i>S. aureus</i>	2	4
Lactic acid bacteria	<i>L. plantarum</i>	0	4	-	11	-	-	29	-
<i>C. perfringens</i>	-	0	-	<i>C. perfringens</i>	3	3	-	3	-
Anaerob. sulph. red	-	0	-	<i>C. perfringens</i>	0	1	-	3	-
Aerobic microorg. in fish products, 20-25 °C	<i>L. plantarum</i> <i>E. coli</i>	0	0	<i>A. hydrophila</i> <i>S. warneri</i> <i>S. aureus</i> <i>S. putrefaciens</i>	0	3	<i>Micrococcus</i> <i>S. aureus</i>	0	19
H ₂ S producing bact. in fish prod.	-	0	-	<i>S. putrefaciens</i>	3	7	-	0	-
Yeasts	<i>K. marxianus</i>	10	10	-	3	-	-	3	-
Moulds	<i>P. verrucosum</i>	4	6	-	2	-	-	3	-

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

Aerobic microorganisms, 30 °C

Mixture A

The colonies counted for this analysis were mainly from the strains of *Lactobacillus plantarum* and *Esherichia coli* present at the highest concentration in mixture A.

Mixture B

Aeromonas hydrophila, *Shewanella putrefaciens*, *Staphylococcus warneri* and *Staphylococcus aureus* formed the colonies counted for this analysis.

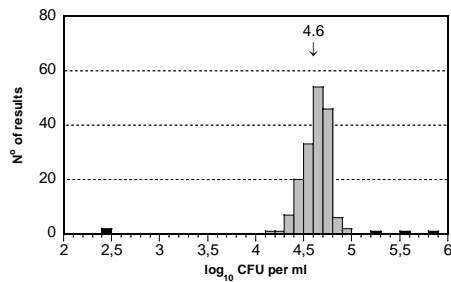
Mixture C

The colonies counted for this analysis were mainly from the strains of *Micrococcus sp.* and *Staphylococcus aureus* present at the highest concentration in mixture C.

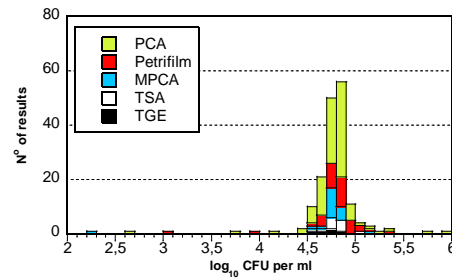
Results of aerobic microorganisms analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	175	4.62	0.13	0	2	3	175	4.78	0.13	0	6	4	174	4.82	0.13	0	3	2
PCA	96	4.62	0.13	0	1	3	96	4.77	0.13	0	3	3	96	4.82	0.12	0	2	2
Petrifilm™	36	4.62	0.13	0	1	0	36	4.82	0.13	0	2	1	35	4.83	0.13	0	1	0
MPCA	20	4.69	0.08	0	0	0	20	4.78	0.11	0	1	0	20	4.84	0.12	0	0	0
TSA	11	4.57	0.13	0	0	0	11	4.78	0.11	0	0	0	11	4.80	0.17	0	0	0
TGE	5	4.55	0.15	0	0	0	5	4.70	0.11	0	0	0	5	4.74	0.12	0	0	0

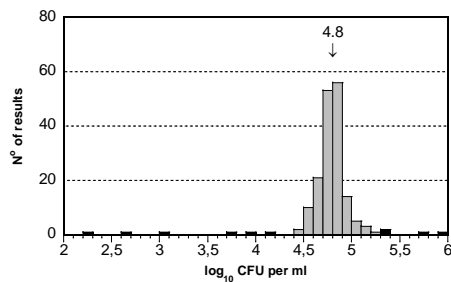
A



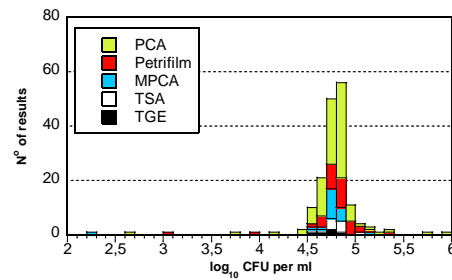
A



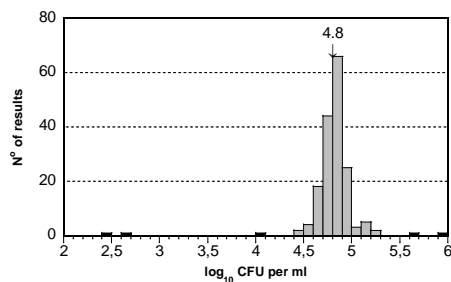
B



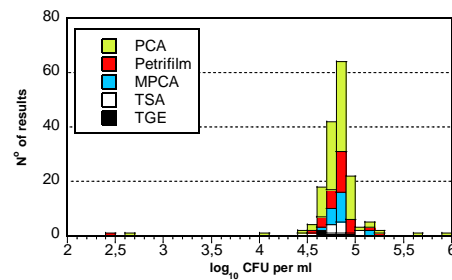
B



C



C



There is no differences in results depending on the medium chosen for the analysis.

Psychrotrophic microorganisms

Mixture A

When controlling mixture A, *Penicillium verrucosum* formed colonies in PCA after 10 days of incubation at 6.5°C. The colonies were however very small and a magnifying glass was used for their enumeration. This can explain part of the false negative results reported for the analysis.

Mixture B

Microorganisms present in mixture B can grow at temperatures lower than 30°C. In our control, after 10 days at 6.5°C colonies were very small and difficult to count without magnifier. Most of the laboratories that reported a false negative result performed an incubation at 6.5 or 7°C.

Mixture C

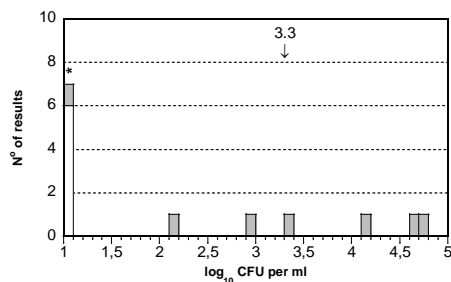
After 10 days of incubation at 6.5°C, we did not observed any growth in PCA during the quality control of mixture C. Similar results were obtained by almost all laboratories that used 6.5 or 7°C as temperature of incubation. However, incubation at higher temperature can allow the growth of microorganisms present in mixture C. Therefore both negative results and counting of cfu should be considered as correct for this analysis. This leads to a very high standard deviation for the results.

Results of psychrotrophic microorganisms analysis

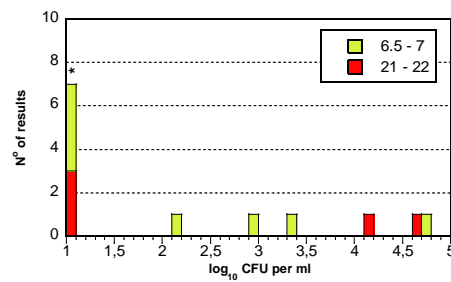
T°C	Mixture A					Mixture B					Mixture C				
	n	m*	s	F	< >	n	m*	s	F	< >	n	m*	s	F	< >
Total	13	3.30	1.38	6	0 0	15	3.08	0.90	7	0 0	12	1.49	2.05	0	0 0
6.5 - 7	8	4.16	-	4	0 0	10	2.87	-	6	0 0	7	0	-	0	0 0
21 - 22	5	3.11	-	2	0 0	5	3.14	-	1	0 0	5	3.42	-	0	0 0

*: median

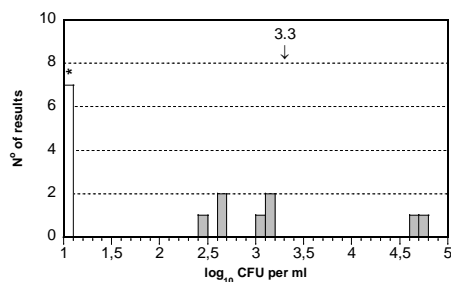
A



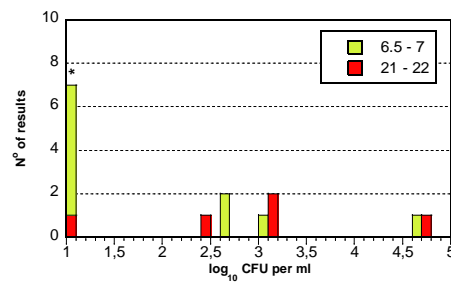
A



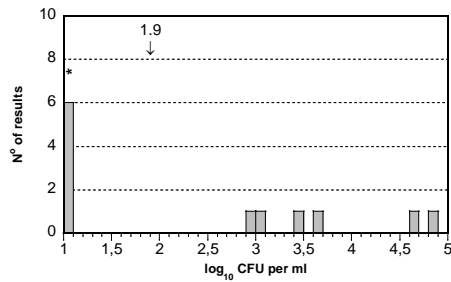
B



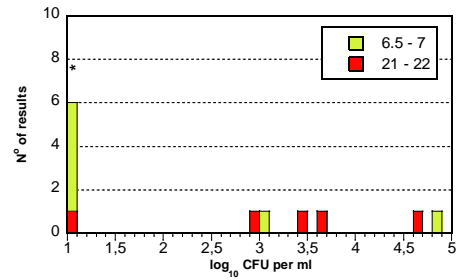
B



C



C



Because of the low amount of results, median instead of mean values are presented in the table. Moreover, no standard deviation was calculated for the results grouped according to the incubation temperature used. Almost all laboratories used PCA or MPCA as medium but the temperature and time of incubation varied depending on the method used. The method NMKL 74:2000 (17°C / 20h + 7°C / 3 days) has been replaced by the method NMKL 86:2013 that prescribes an incubation at 6.5°C / 10 days or 17°C / 20h + 7°C / 3 days. There are 3 ISO methods for the enumeration of psychrotrophic microorganisms: ISO 17410:2001 with an incubation at 6.5°C / 10 days and, ISO 6730:2005 and ISO 8552:2004, both specific for milk, with an incubation at 6.5°C / 10 days and 21°C / 24h, respectively. This variation reflects the different definition that laboratories have of psychrotrophic microorganisms and makes the statistical evaluation of the results quite difficult.

Enterobacteriaceae and *Escherichia coli*

Mixture A

A strain of *Escherichia coli* was target-organism for these analyses.

Mixture B

There was no target-organism for these analyses. However, 64 false positive results were reported for the analysis of enterobacteriaceae (out of 150 results). *Aeromonas hydrophila* forms red colonies on VRBG but is oxidase-positive and therefore differentiates from enterobacteriaceae that are oxidase negative.

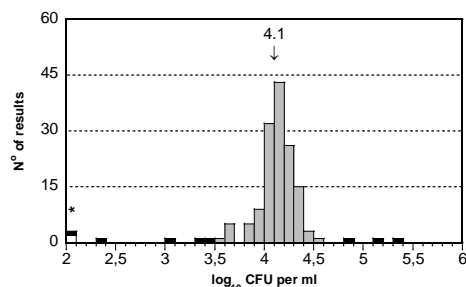
Mixture C

A strain of *Escherichia coli* was target-organism for these analyses.

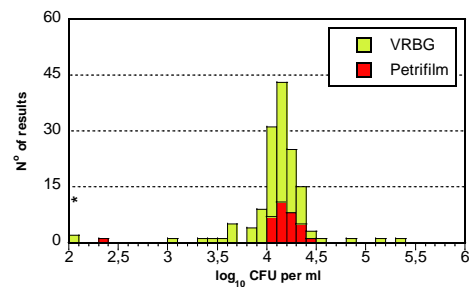
Results of Enterobacteriaceae analysis

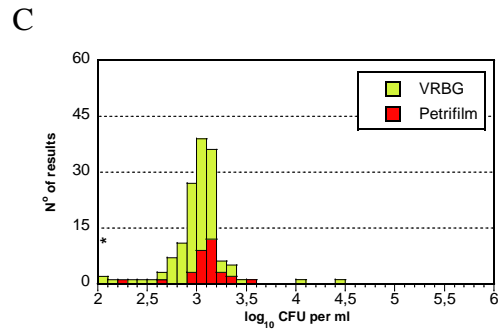
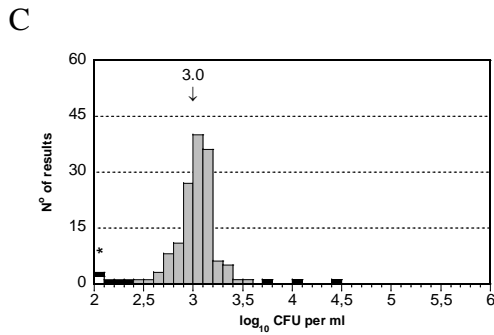
Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	150	4.12	0.16	2	5	3	150	-	-	64	-	-	149	3.02	0.17	2	4	3
VRBG	113	4.11	0.17	1	4	3	112	-	-	36	-	-	113	3.00	0.16	1	3	2
Petrifilm™ Entero	33	4.18	0.11	0	1	0	33	-	-	26	-	-	32	3.11	0.16	0	1	0

A



A



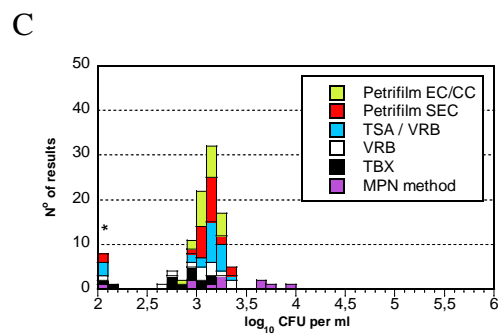
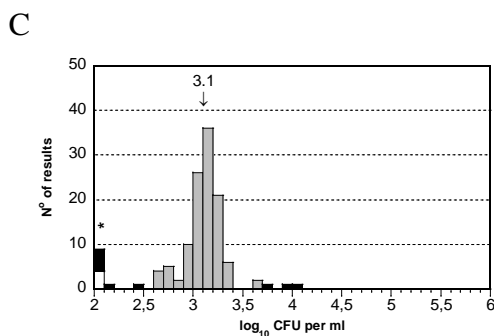
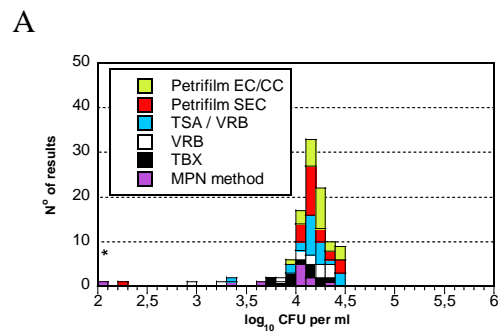
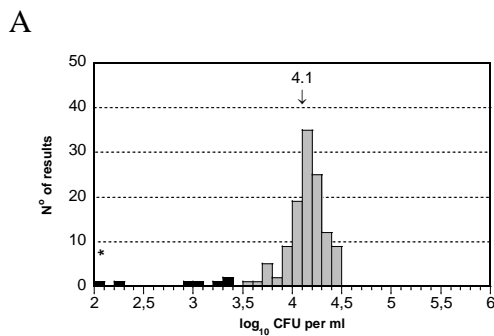


Most of the laboratories used VRBG plates or Petrifilm™ Enterobacteriaceae as medium and similar average values were obtained, both for mixture A and C.

For mixture B, 79% of the laboratories that used Petrifilm™ and 32 % of those that used VRBG reported a false positive results. However, the false positive results are linked to the absence of confirmation step which occurs more often when Petrifilm™ is chosen.

Results of *E.coli* analysis

Medium/method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	125	4.14	0.18	0	7	0	124	-	-	0	-	-	126	3.09	0.18	4	7	3
Petrifilm™ EC/CC	24	4.22	0.12	0	0	0	24	-	-	1	-	-	23	3.10	0.11	1	0	0
Petrifilm™ SEC	24	4.19	0.12	0	1	0	22	-	-	0	-	-	24	3.12	0.09	3	1	0
TSA/VRB	23	4.18	0.12	0	1	0	22	-	-	0	-	-	23	3.14	0.09	0	0	0
VRB	13	4.18	0.15	0	2	0	13	-	-	1	-	-	13	3.06	0.20	0	1	0
TBX	13	4.04	0.17	0	0	0	13	-	-	0	-	-	13	2.93	0.13	0	2	0
MPN-method	11	4.05	0.18	0	2	0	11	-	-	0	-	-	11	3.26	0.26	0	1	2



The same strain of *E. coli* was target-organism in mixture A and C. Results are distributed similarly in both cases, with a tail of lower values that is not possible to link to the use of any method or medium.

Presumptive *Bacillus cereus*

Mixture A

There was no target-organism for this analysis in mixture A.

Mixture B

There was no target-organism for this analysis. On blood agar, some atypical colonies were surrounded by a haemolytic zone and on Bcsa, *Aeromonas hydrophila* formed light blue colonies without precipitation zone. 10 of the laboratories that reported a false positive result used only BA or BA-P when performing the analysis.

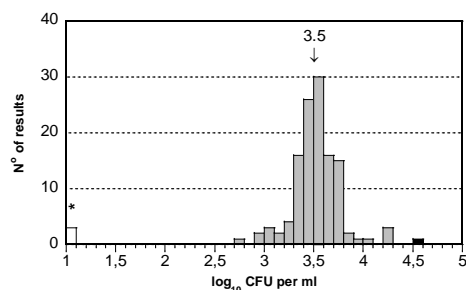
Mixture C

Mixture C contained a typical strain belonging to the *Bacillus cereus* group and similar average values were obtained independently of the media used

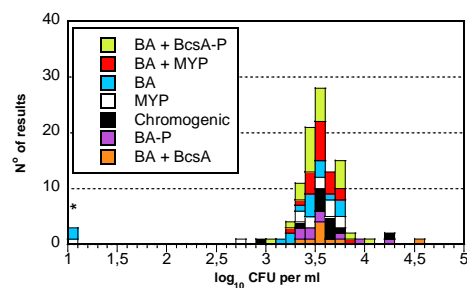
Results of presumptive *B. cereus* analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	123	-	-	1	-	-	124	-	-	17	-	-	126	3.52	0.23	3	0	1
BA + BcsA-P	25	-	-	0	-	-	25	-	-	1	-	-	26	3.52	0.20	0	0	0
BA + MYP	20	-	-	0	-	-	20	-	-	1	-	-	20	3.56	0.14	0	0	0
BA	16	-	-	0	-	-	16	-	-	3	-	-	17	3.48	0.19	2	0	0
MYP	13	-	-	0	-	-	13	-	-	1	-	-	13	3.50	0.25	1	0	0
Chromogenic	12	-	-	1	-	-	12	-	-	0	-	-	12	3.58	0.27	0	0	0
BA-P	9	-	-	0	-	-	9	-	-	7	-	-	9	3.61	0.29	0	0	0
BA + BcsA	8	-	-	0	-	-	9	-	-	3	-	-	9	3.55	0.12	0	0	1

C



C



Coagulase-positive *Staphylococci*

Mixture A

There was no target-organism for this analysis in mixture A.

Mixture B

Strains of *Staphylococcus warneri* and *Staphylococcus aureus* were included in mixture B. Only the latter was target-organism for this analysis. On BP-agar with RPF (Rabbit plasma fibrinogen), colonies of *S. warneri* were atypical, without precipitation zone. On BP-agar, they were smaller than those of *S. aureus* and negative when further tested for coagulase activity.

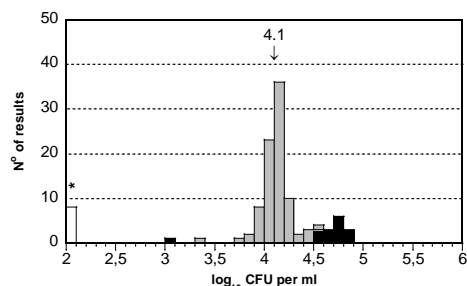
Mixture C

A strain of *Staphylococcus aureus* was target-organism for this analysis.

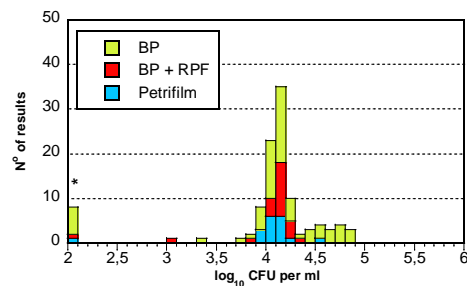
Results of coagulase-positive *Staphylococci* analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	113	-	-	0	-	-	111	4.11	0.16	8	1	15	112	4.62	0.10	2	4	0
BP	68	-	-	0	-	-	66	4.10	0.19	6	0	12	67	4.63	0.09	2	4	0
BP + RPF	24	-	-	0	-	-	24	4.14	0.11	1	1	0	24	4.64	0.09	0	0	0
Petrifilm™ Staph	18	-	-	0	-	-	18	4.07	0.08	1	0	1	18	4.53	0.10	0	0	0

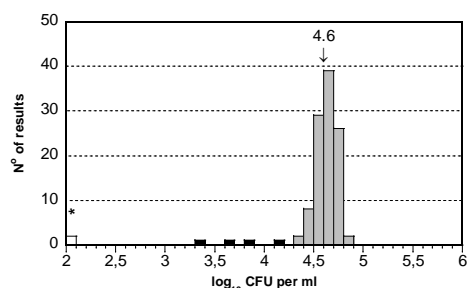
B



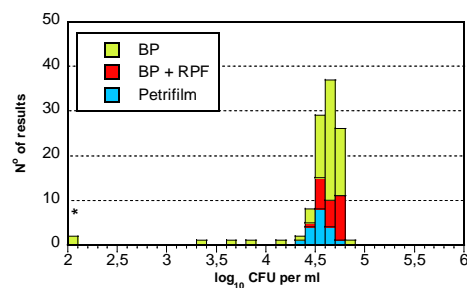
B



C



C



Almost all high outliers results of mixture B are linked to the use of BP-agar. On this medium, the coagulase reaction is not tested and colonies of *S. warneri* can be misjudged as coagulase-positive staphylococci. However, laboratories that used BP-agar reported that they performed a confirmation step, which suggests that only *S. aureus* colonies were confirmed and/or that the confirmation test failed.

Results obtained with Petrifilm™ were slightly lower than the total average (although not significantly). In this case, colonies were counted after 1 day of incubation instead of 2 days when using traditional plates. This could lead to smaller colonies, increasing the difficulty of enumeration and resulting in a lower amount of counted colonies.

Lactic acid bacteria

Mixture A

A strain of *Lactobacillus plantarum* was target-organism for this analysis.

Mixture B

There was no target-organism for this analysis in mixture B but 7 laboratories reported a false positive result. Both *Staphylococcus warneri* and *Staphylococcus aureus* can form small colonies on MRS and pinpoint colonies on MRS-aB.

Mixture C

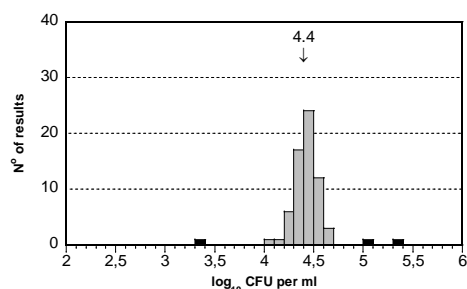
There was no target-organism for this analysis; however, 19 of the 66 laboratories that performed the analysis reported the presence of lactic acid bacteria in mixture C. *Staphylococcus aureus* can form small colonies on MRS and pinpoint colonies on

MRS-aB. Lactic acid bacteria grow well on MRS-aB, forming white or grey colonies with a diameter of 1.5 ± 0.5 mm after 5 days of incubation at 25° C in anaerobiosis. Moreover *S. aureus* is catalase positive while lactic acid bacteria are catalase negative.

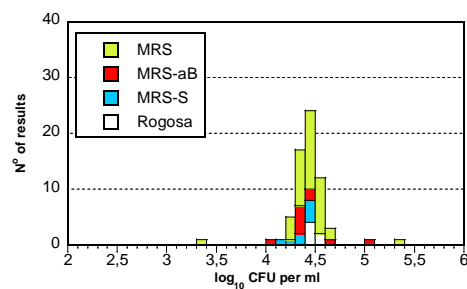
Results of lactic acid bacteria analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	67	4.41	0.11	0	1	2	66	-	-	7	-	-	66	-	-	19	-	-
MRS	42	4.43	0.10	0	1	1	42	-	-	5	-	-	42	-	-	12	-	-
MRS-aB	10	4.38	0.13	0	0	1	9	-	-	1	-	-	9	-	-	5	-	-
MRS-S	8	4.36	0.12	0	0	0	8	-	-	1	-	-	8	-	-	1	-	-
Rogosa	6	4.46	0.04	0	0	0	6	-	-	0	-	-	6	-	-	0	-	-

A



A



The enumeration of *L. plantarum* in mixture A did not cause any difficulties and all media gave similar results.

For the analysis of mixture B and mixture C, false positive results are linked to the use of MRS and MRS-aB which are the media recommended in the method ISO 15214:1998 and NMKL 140.2007, respectively. This suggests that these media might be less selective than MRS-S and Rogosa, and allow the growth of the microorganisms present in the mixtures.

C. perfringens and anaerobic sulphite-reducing bacteria

Mixture A

There was no target-organism for these analyses in mixture A.

Mixture B

A strain of *Clostridium perfringens* was target-organism for both analyses.

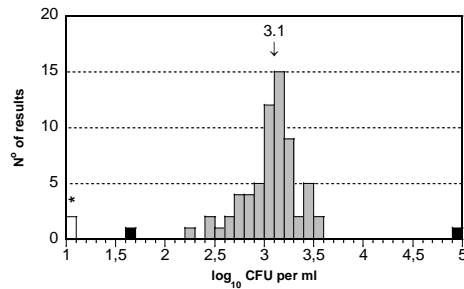
Mixture C

There was no target-organism for these analyses in mixture C.

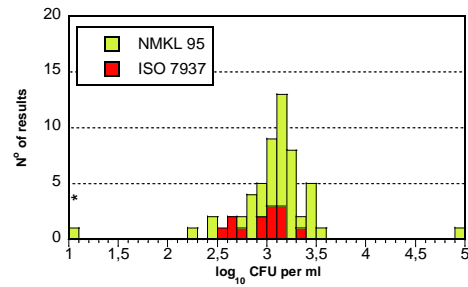
Results of *C. perfringens* analysis

Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	67	-	-	0	-	-	68	3.06	0.26	2	1	1	68	-	-	2	-	-
NMKL 95:2009	43	-	-	0	-	-	44	3.09	0.26	0	1	1	43	-	-	1	-	-
ISO 7937:2004	13	-	-	0	-	-	13	2.95	0.22	0	0	0	14	-	-	0	-	-

B



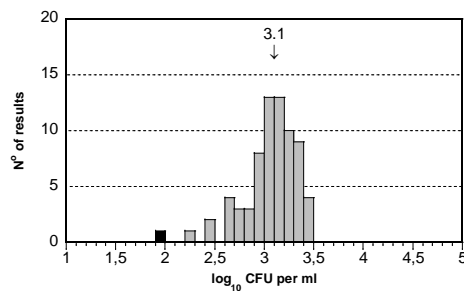
B



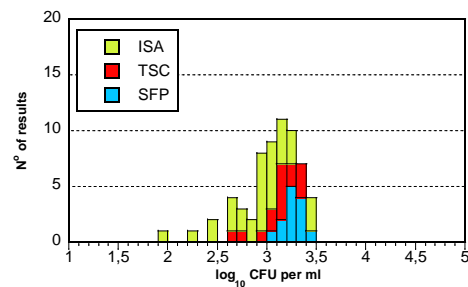
Results of anaerobic sulphite-reducing bacteria analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	70	-	-	0	-	-	71	3.06	0.25	0	1	0	71	-	-	1	-	-
ISA	33	-	-	0	-	-	34	2.96	0.28	0	1	0	34	-	-	1	-	-
TSC	15	-	-	0	-	-	15	3.10	0.20	0	0	0	15	-	-	0	-	-
SFP	12	-	-	0	-	-	13	3.26	0.11	0	0	0	30	-	-	0	-	-

B



B



These analyses did not cause any difficulties and results for mixture B are approximately the same regardless of the method used. For the analysis of *C. perfringens*, almost all laboratories used TSC medium, and the method NMKL 95:2009 or EN ISO 7937:2004. The first method describes an incubation at 37 °C for 24h, while the second at 35 or 37 °C for 20h.

For the analysis of anaerobic sulphite-reducing bacteria, slightly higher results were obtained with the use of SFP. It has been shown that SFP agar is less selective than TSC agar but also allows a slightly higher rate of recovery of *C. perfringens* than TSC (2). Moreover, at NFA, we have noticed that the strain of *C. perfringens* present in mixture B had a lower recovery on TSC agar with a pH higher than 7.6.

However, both methods NMKL 56:2008 and ISO 15213:2003 for the analysis of anaerobic sulphite-reducing bacteria in food prescribe the use of ISA and not TSC or SFP which are dedicated to the analysis of *C. perfringens*.

Last, it should be noted that the method NMKL 56 has been revised and is now called "Sulphite-reducing clostridia. Determination in food". This 5th edition of the method describes the determination of the number of anaerobic sulphite-reducing bacteria, as previously, but includes also the possible determination of sulphite-reducing clostridia by further confirmation.

Aerobic microorganisms and H₂S producing bacteria in fish

Mixture A

As for the analysis of aerobic microorganisms at 30 °C, colonies counted here were from the strains of *Lactobacillus plantarum* and *Esherichia coli*. The mixture did not contain any H₂S producing bacteria.

Mixture B

Colonies of *Aeromonas hydrophila*, *Shewanella putrefaciens*, *Staphylococcus warneri* and *Staphylococcus aureus* formed the colonies counted for the analysis of aerobic microorganisms. Only the strain of *Shewanella putrefaciens*, which form black colonies on iron agar, was target-organism for the analysis of H₂S producing bacteria.

Mixture C

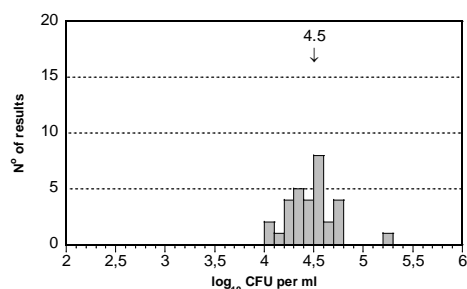
For the analysis of aerobic microorganisms, colonies counted were mainly from the strains of *Micrococcus spp.* and *Staphylococcus aureus*. The mixture did not contain any H₂S producing bacteria.

Results of aerobic microorganisms in fish products analysis

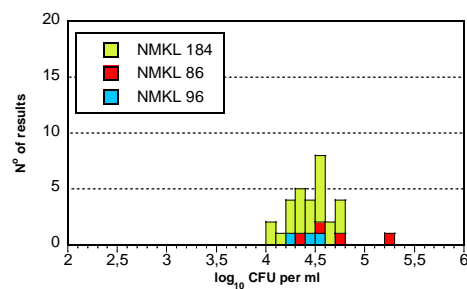
Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	31	4.46	0.23	0	0	0	31	4.15	0.33	0	1	0	31	4.75	0.11	0	5	1
NMKL 184:2006	24	4.43	0.19	0	0	0	24	4.11	0.31	0	0	0	24	4.77	0.10	0	4	0
NMKL 86:2013	4	4.62*	-	0	0	0	4	4.71*	-	0	1	0	4	4.58*	-	0	0	1
NMKL 96:2003	2	4.46*	-	0	0	0	3	4.15*	-	0	0	0	3	4.74*	-	0	1	0

* median

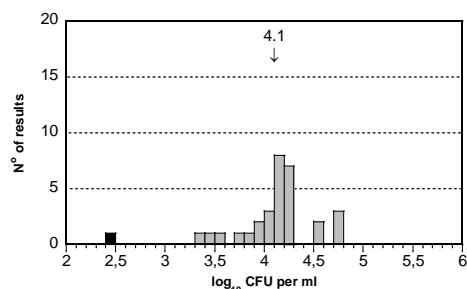
A



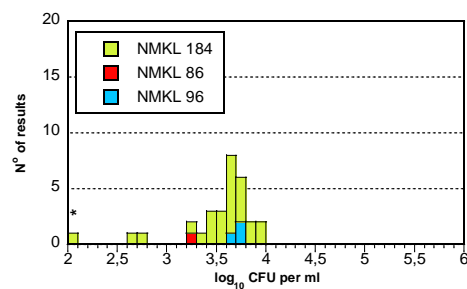
A



B

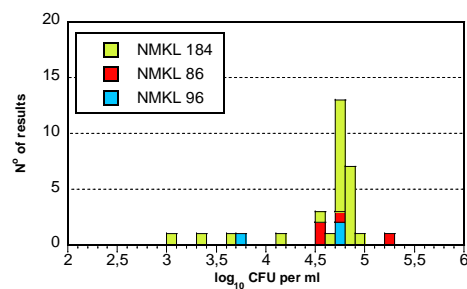
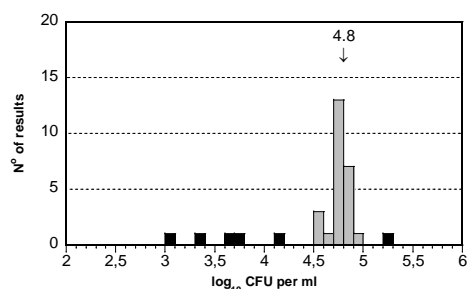


B



C

C

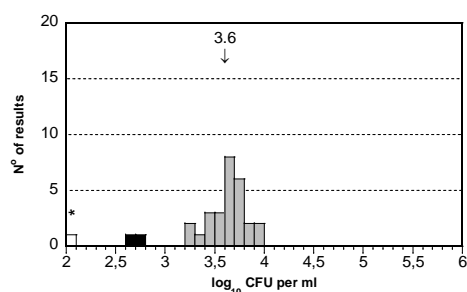


Results of H_2S producing bacteria in fish products analysis

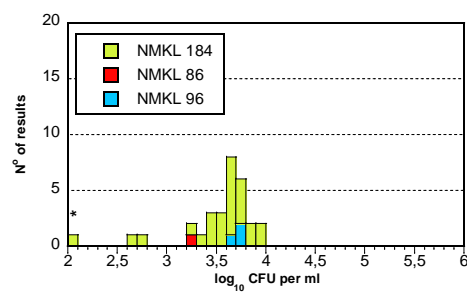
Method	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	29	-	-	0	-	-	30	3.62	0.18	1	2	0	29	-	-	0	-	-
NMKL 184:2006	25	-	-	0	-	-	26	3.62	0.17	1	2	0	25	-	-	0	-	-
NMKL 96:2003	3	-	-	0	-	-	3	3.71*	-	0	0	0	3	-	-	0	-	-

* median

B



B



Most of the laboratories that performed both analyses used iron agar and the method NMKL 184:2006 “Aerobic count and specific spoilage organisms in fish and fish products”. The method NMKL 96 describes the analyses of coliform bacteria, thermotolerant coliform bacteria and *E. coli* by MPN methods for fresh and frozen seafood while the method NMKL 86 describes the determination of aerobic microorganisms in foods. These two methods are not adapted to the analyses performed here.

Yeasts and moulds

Mixture A

Mixture A contained a strain of *Kluyveromyces marxianus* that was target-organism for the analysis of yeasts. Out of 147 laboratories that performed the analysis, 15 reported an absence of yeast in the mixture and 13 reported a result that was identified as high outlier. The false negative results could be explained by the low concentration of yeast in mixture A ($1.89 \log_{10} \text{cfu ml}^{-1}$ in our quality control). A strain of *Penicillium verrucosum* was target-organism for the analysis of moulds.

Mixture B

Mixture B contained neither yeast nor mould.

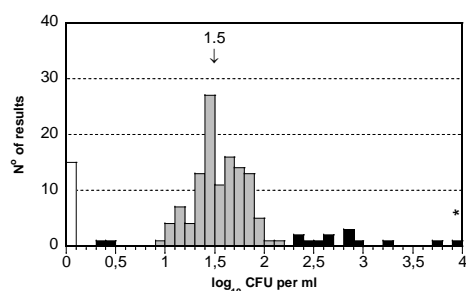
Mixture C

Mixture C contained neither yeast nor mould.

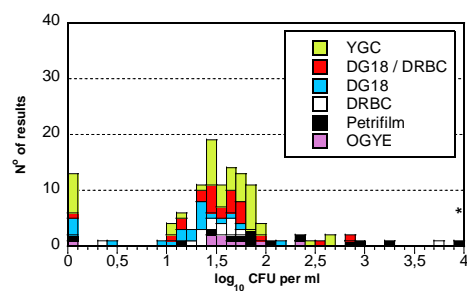
Results of yeasts analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	147	1.53	0.25	15	2	13	142	-	-	4	-	-	143	-	-	5	-	-
YGC	45	1.60	0.22	7	0	3	45	-	-	1	-	-	45	-	-	0	-	-
DG18/DRBC	24	1.51	0.23	1	0	2	22	-	-	0	-	-	23	-	-	0	-	-
DG18	20	1.36	0.29	3	1	0	19	-	-	0	-	-	19	-	-	0	-	-
DRBC	14	1.50	0.18	0	1	1	14	-	-	0	-	-	13	-	-	1	-	-
Petrifilm™	14	1.70	0.25	1	0	5	12	-	-	2	-	-	12	-	-	1	-	-
OGYE	9	1.60	0.15	1	0	1	9	-	-	1	-	-	9	-	-	1	-	-

A



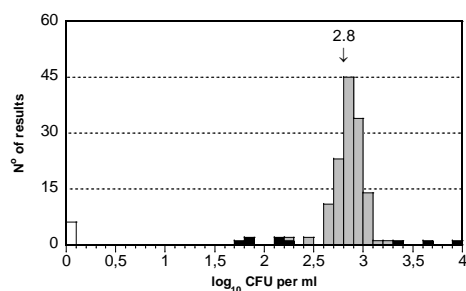
A



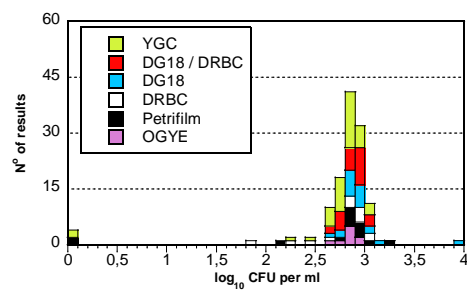
Results of moulds analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	147	2.85	0.13	6	6	3	142	-	-	3	-	-	142	-	-	4	-	-
YGC	42	2.80	0.14	2	0	0	42	-	-	1	-	-	42	-	-	1	-	-
DG18/DRBC	26	2.85	0.11	0	0	0	24	-	-	0	-	-	25	-	-	0	-	-
DG18	20	2.86	0.11	0	0	1	19	-	-	1	-	-	19	-	-	1	-	-
DRBC	13	2.85	0.16	0	2	0	13	-	-	0	-	-	13	-	-	0	-	-
Petrifilm™	15	2.92	0.13	2	1	0	13	-	-	0	-	-	13	-	-	1	-	-
OGYE	9	2.84	0.10	0	0	0	9	-	-	0	-	-	9	-	-	0	-	-

A



A



Most of the laboratories performed yeast and mould analyses according to the methods NMKL 98:2005 and ISO 21527:2008 which describes the use of DRBC, DG18 and/or OGYE, or according to the method ISO 6811:2004 / IDF:94:2004 which describes the use of YGC or OGYE.

The analysis of moulds did not cause any difficulties and results are very similar regardless of the medium used.

For the analysis of yeast, the false negative results reported for mixture A are mainly linked to the use of YGC and DG18. During our quality control we observed that colonies of *K. marxianus* were bigger and easier to count on DRBC than on DG18. We did not perform the analysis on YGC. There is no link between the high outliers results and the medium/method used for the analysis of yeast for mixture A.

Outcome of the results of individual laboratory - assessment

In order to allow comparison of the results from different analyses and mixtures, all the results of the analyses were 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. For qualitative analyses, a z-score of zero is attributed for a correct answer. The z-scores obtained, which are listed in Annex 2, can be used as a tool by laboratories when following up on the results.

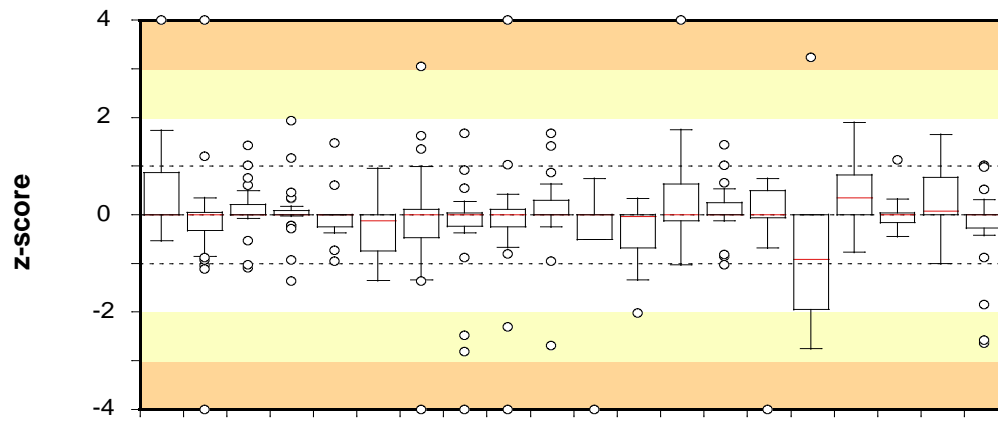
All the results from each laboratory – outliers included and false results excluded – were compiled into a box plot based on their z-scores. The smaller and more centred round zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

The laboratories were not grouped or ranked based on their results. However, for each laboratory, the numbers of false results and outliers are presented below the box plots. These results are also highlighted in Annex 1, where all the reported results are listed, and the minimum and maximum accepted values for each analysis are stated.

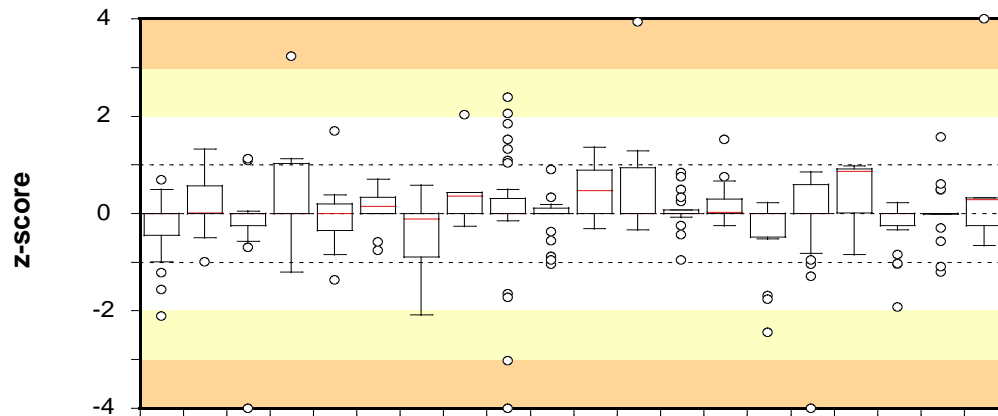
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (3). Samples for follow-up can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

Box plots and numbers of deviating results for each laboratory

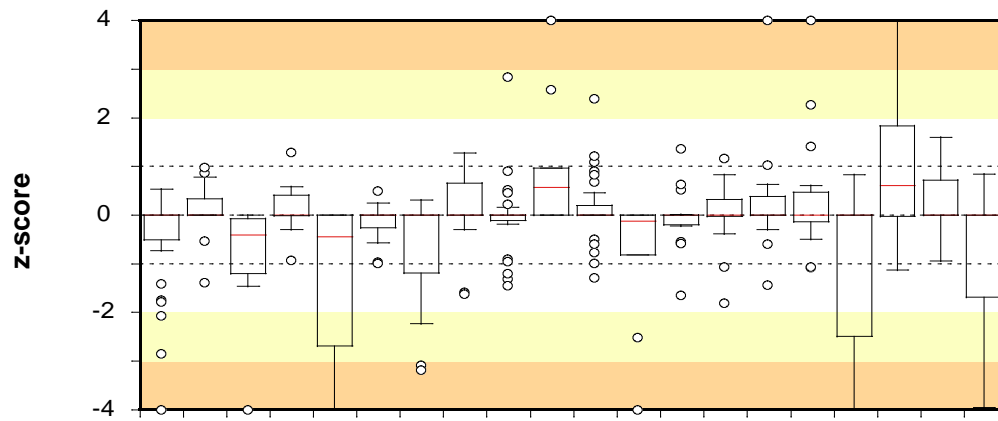
- *The plots are based on the laboratory results from all analyses transformed into z-scores calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation.*
- *Correct results for quantitative analyses without target organism and for qualitative analyses 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.*
- *Very deviating results are represented by circles and are calculated as follow: the lowest result in the box $- 1.5 \times$ (the highest result in the box $-$ the lowest result in the box) or the highest result in the box $+ 1.5 \times$ (the highest result in the box $-$ the lowest result in the box). z-scores higher than +4 and less than -4 are positioned at +4 and -4 , respectively, in the plot.*
- *The background is divided by lines and shaded fields to indicate ranges in order to simplify location of laboratory results.*



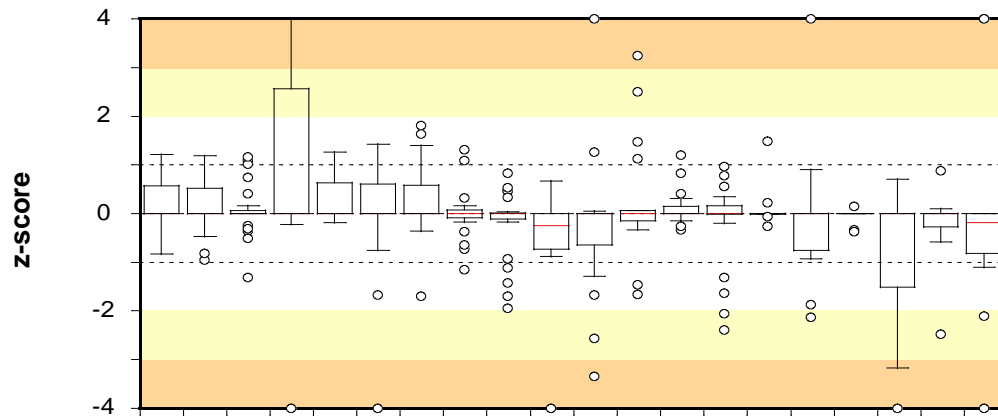
Lab no	1149	1237	1594	1970	2035	2058	2072	2324	2344	2386	2402	2458	2459	2637	2642	2670	2704	2720	2745	2764
No. of results	19	28	27	37	18	11	32	20	30	18	9	18	13	30	19	8	19	11	18	20
False positive	2	-	-	-	-	1	1	3	-	-	1	1	3	-	1	-	2	1	-	1
False negative	-	2	-	2	-	-	-	1	1	-	-	1	-	-	1	1	-	-	-	-
Low outliers	-	1	-	-	-	-	1	1	1	-	2	-	-	-	1	-	-	-	-	-
High outliers	1	1	-	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-



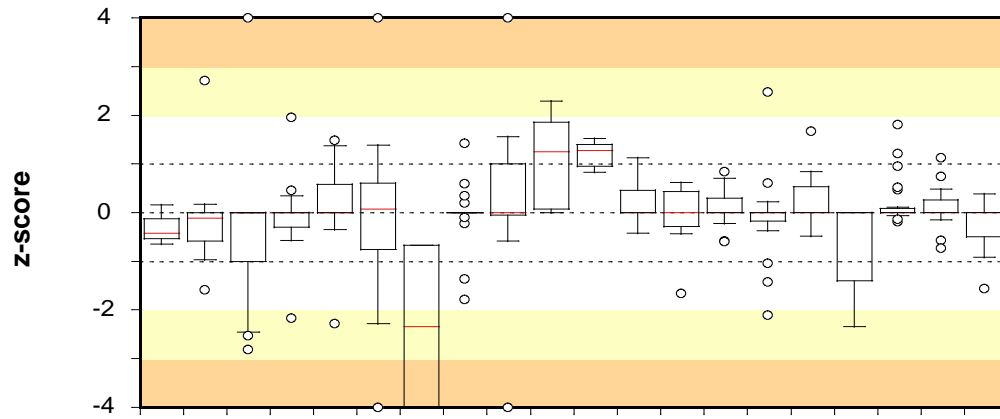
Lab no	2842	2920	2941	3055	3126	3159	3225	3243	3305	3457	3533	3543	3587	3626	3831	3868	3925	4047	4050	4064
No. of results	23	12	24	14	9	17	14	6	34	24	6	17	21	18	15	36	3	19	18	5
False positive	1	-	1	1	-	1	1	-	2	-	-	1	-	-	-	-	-	1	-	1
False negative	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Low outliers	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1



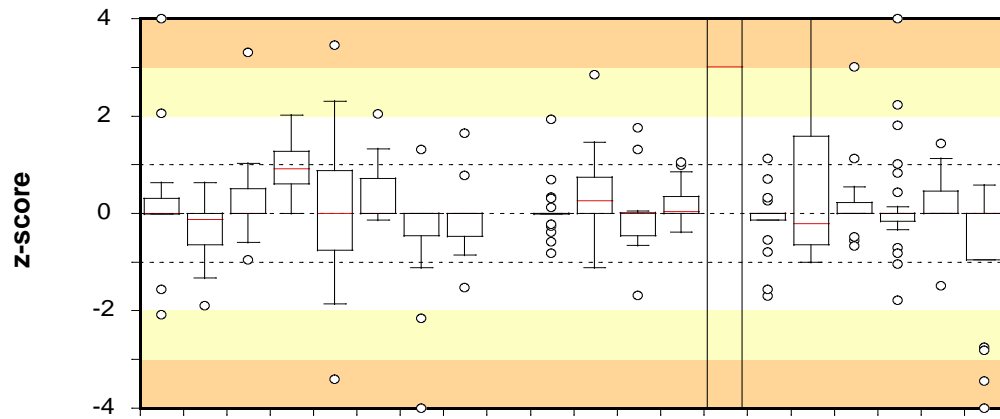
Lab no	4100	4171	4246	4266	4278	4288	4305	4339	4352	4400	4562	4564	4635	4664	4817	4840	4873	4879	4889	4951
No. of results	33	22	13	17	14	26	20	36	35	10	30	10	24	23	21	18	16	11	24	14
False positive	-	1	2	1	1	-	-	-	1	1	-	1	-	1	-	3	1	1	-	1
False negative	-	1	-	-	-	1	1	-	-	1	-	1	-	-	-	-	1	-	-	-
Low outliers	1	-	1	-	3	-	-	-	-	-	-	1	-	-	-	-	1	-	-	1
High outliers	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	-	1	-	-



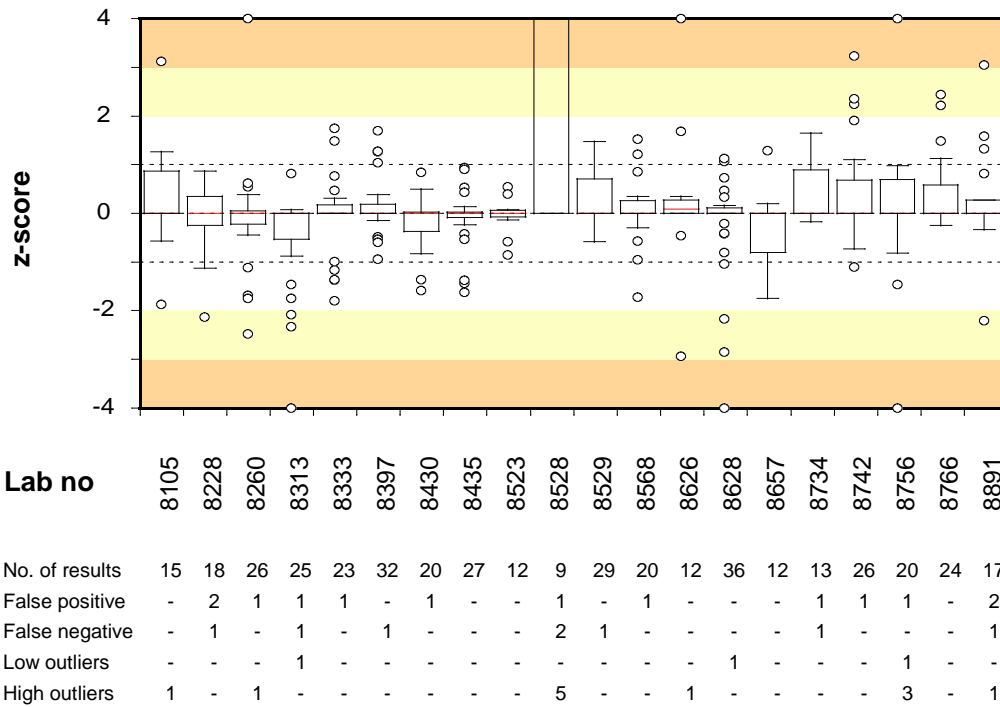
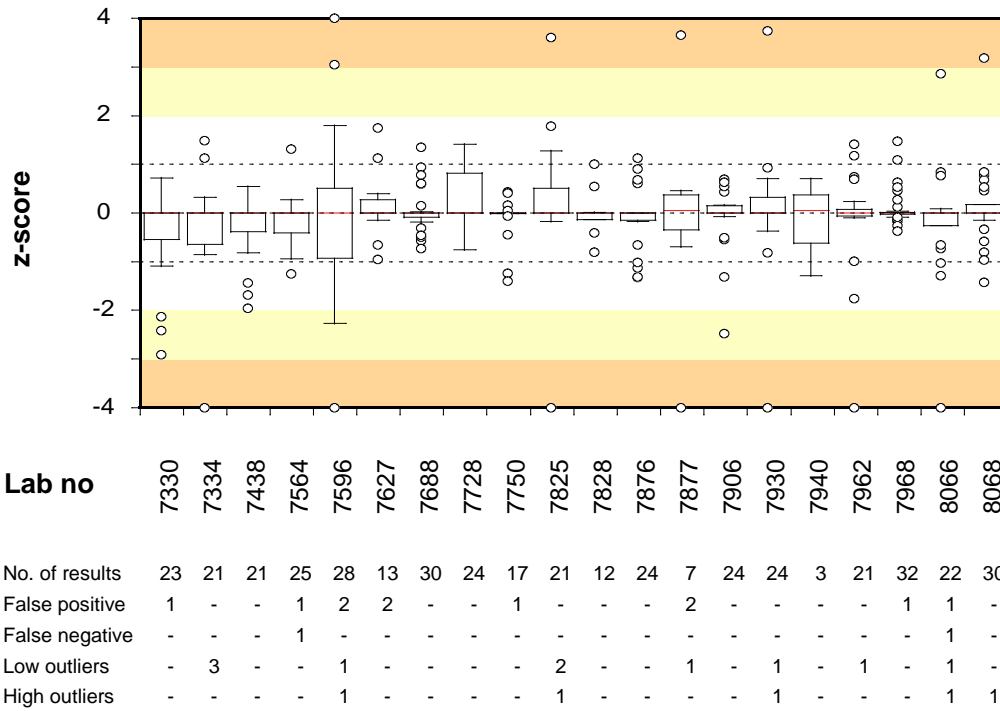
Lab no	4955	4980	5018	5100	5119	5120	5200	5201	5204	5220	5250	5304	5329	5333	5338	5352	5545	5553	5615	5632
No. of results	30	26	29	12	10	35	18	19	28	9	15	18	20	23	9	23	11	17	24	16
False positive	-	1	1	1	-	1	-	1	3	-	-	-	1	1	-	-	-	-	-	1
False negative	-	-	-	2	2	-	-	1	2	-	-	-	-	-	-	1	4	-	-	-
Low outliers	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	1	-	1
High outliers	-	-	-	3	-	-	-	-	-	-	1	1	-	-	-	1	-	-	-	2

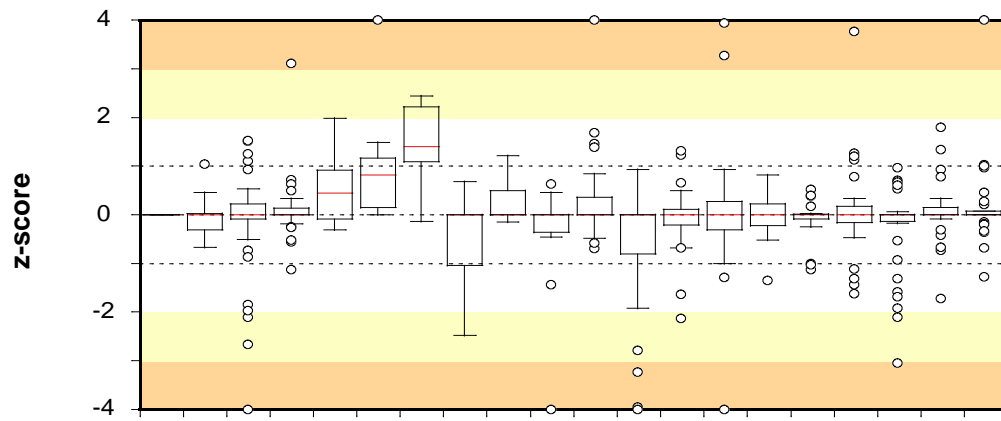


Lab no	5701	5801	5808	5883	5933	5950	5993	6109	6175	6224	6232	6253	6258	6343	6352	6368	6443	6456	6490	6594
No. of results	3	12	13	24	23	18	2	18	12	7	5	24	12	24	26	26	6	27	20	17
False positive	-	3	1	-	1	1	1	-	-	1	1	-	2	-	-	1	-	-	1	1
False negative	-	-	1	-	-	-	-	-	-	1	-	-	1	-	1	-	-	-	-	-
Low outliers	-	-	-	-	-	2	1	-	1	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-

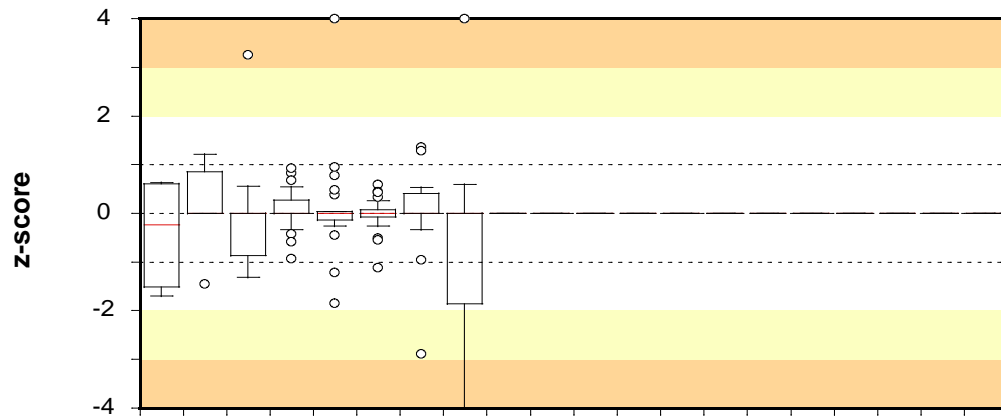


Lab no	6647	6658	6686	6762	6852	6885	6944	6958	6971	6992	7024	7096	7182	7191	7207	7232	7242	7248	7253	7282
No. of results	11	14	24	8	11	21	15	12	5	22	14	15	14	9	18	6	18	29	14	18
False positive	-	1	-	1	2	3	-	2	1	-	1	-	3	1	-	3	-	1	-	-
False negative	-	-	-	-	2	-	-	1	-	-	-	-	1	2	-	-	-	-	1	-
Low outliers	-	-	-	-	-	-	1	-	5	-	-	-	-	3	-	-	-	-	-	1
High outliers	1	-	1	-	1	-	-	-	-	-	-	-	-	3	-	1	-	1	-	-





Lab no	8909	8918	8955	9002	9003	9034	9078	9217	9408	9429	9436	9441	9451	9453	9512	9555	9559	9569	9589	9662
No. of results	-	16	36	27	8	15	5	17	15	27	31	37	24	19	14	19	24	35	27	30
False positive	-	2	-	-	-	-	1	1	-	-	1	-	-	2	1	3	3	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-	2	-	1	-	-
Low outliers	-	-	1	-	-	-	-	-	-	4	-	2	-	1	-	-	-	-	-	-
High outliers	-	-	-	1	-	1	-	-	-	-	1	-	-	1	-	-	-	-	-	1



Lab no	9747	9783	9853	9886	9890	9903	9923	9950
No. of results	6	9	14	31	22	24	17	14
False positive	-	-	1	1	2	-	1	1
False negative	-	-	-	1	-	-	-	-
Low outliers	-	-	-	-	-	-	-	1
High outliers	-	-	-	-	1	-	-	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 (4). Before analysing the samples, the contents of each vial had to be dissolved in 254 ml of diluent. The organisms present in the mixtures are listed in Table 2.

Table 2. *Microorganisms present in mixture A-C supplied to participants*

Mixture ¹	Microorganism	Strain no.
A	<i>Lactobacillus plantarum</i>	SLV-445
	<i>Escherichia coli</i>	SLV-524
	<i>Kluyveromyces marxianus</i>	SLV-439
	<i>Penicillium verrucosum</i>	SLV-526
B	<i>Aeromonas hydrophila</i>	SLV-454
	<i>Clostridium perfringens</i>	SLV-442
	<i>Staphylococcus warneri</i>	SLV-565
	<i>Staphylococcus aureus</i>	SLV-350
	<i>Shewanella putrefaciens</i>	SLV-520
C	<i>Micrococcus sp.</i>	SLV-055
	<i>Escherichia coli</i>	SLV-524
	<i>Bacillus cereus</i>	SLV-518
	<i>Staphylococcus aureus</i>	SLV-280

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

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 (I_2) do not exceed simultaneously 2.6 and 2.0, respectively.

Table 3. Concentration mean (*m*) and standard deviation (*s*) from the quality control of the mixtures, expressed in \log_{10} cfu (colony forming units) per ml of sample.

Analysis and method	A		B		C	
	m	s	m	s	m	s
Aerobic microorganisms, 30 °C NMKL method no. 86	4.87	0.12	4.79	0.07	4.88	0.03
Psychrotrophic microorganisms NMKL method no. 86	2.95	0.06	3.19	0.09	–	–
Enterobacteriaceae NMKL method no. 144	4.33	0.04	–	–	3.15	0.04
<i>Escherichia coli</i> NMKL method no. 125	4.34	0.04	–	–	3.25	0.04
Presumptive <i>Bacillus cereus</i> NMKL method no. 67	–	–	–	–	3.54	0.10
Coagulase-positive staphylococci NMKL method no. 66	–	–	4.21	0.04	4.70	0.06
Lactic acid bacteria NMKL method no. 140	4.54	0.09	–	–	–	–
<i>Clostridium perfringens</i> NMKL method no. 95	–	–	3.10	0.04	–	–
Anaerobic sulphite-reducing bacteria NMKL method no. 56	–	–	3.11	0.06	–	–
Aerobic microorganisms in fish products NMKL method no. 184, IA	4.77	0.06	4.62	0.05	4.91	0.04
H ₂ S-producing bacteria in fish products NMKL method no 184, IA	–	–	4.05	0.09	–	–
Yeasts NMKL method no. 98, DG18	1.89	0.16	–	–	–	–
Moulds NMKL method no. 98, DG18	3.05	0.05	–	–	–	–

– No target-organism

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58-64.
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3. Anonymous, 2012. Protocol. Microbiology. Drinking Water & Food. The National Food Agency.
4. 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.

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.
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Information available on our website: www.livsmedelsverket.se/en/RM-micro