

Proficiency testing

Food Microbiology

– April 2013

by Laurence Nachin, Christina Normark, Irina Boriak and Ingela Tillander



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 used by laboratories 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: www.slv.se/absint

The National Food Agency's reference material

As a complement to the proficiency testing, National Food Agency also produces reference material (RM) for internal quality control: a total of 7 RM for food and drinking water microbiological analyses, including pathogens, are available.

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

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Proficiency Testing

Microbiology – Food

April 2013



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

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Abbreviations

Media

BA	Blood agar
BcS	Bacillus cereus Selective agar
BP	Baird-Parker agar
BP+RPF	Baird-Parker agar with Rabbit Plasma Fibrinogen
Chrom .	Chromogenic medium
DG 18	Dichloran Glycerol agar
DRBC	Dichloran Rose Bengal Chloramphenicol agar
IA	Iron agar
ISA	Iron sulphite agar
MPCA	Milk Plate Count agar
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 B
PCA	Plate Count Agar
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

EN	European standard from the Comité Européen de Normalisation (CEN)
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. many laboratories choose a medium that differs from that in the reported standard methods. 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.

Tables and figures legend

Tables (not Table 1)

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

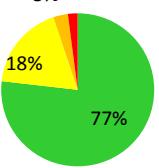
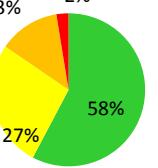
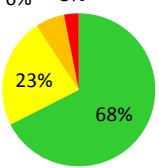
Results of the PT round April 2013

General outcome

Samples were sent to 202 laboratories, 46 in Sweden, 139 in other European countries, and 17 outside Europe. 194 laboratories reported results, 110 (57 %) provided at least one result that received an annotation. In the previous round (April 2012) with similar analyses, the proportion was 53 %.

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

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

	Mixture A			Mixture B			Mixture C		
% participants with	 0 annotation: 77% 1 annotation: 18% 2 annotations: 3% >2 annotations: 2%			 0 annotation: 58% 1 annotation: 27% 2 annotations: 13% >2 annotations: 2%			 0 annotation: 68% 1 annotation: 23% 2 annotations: 6% >2 annotations: 3%		
Organisms	<i>Hafnia alvei</i> <i>Bacillus cereus</i> <i>Lactobacillus plantarum</i> <i>Penicillium verrucosum</i> <i>Aspergillus sp.</i>			<i>Aeromonas hydrophila</i> <i>Clostridium perfringens</i> <i>Staphylococcus warneri</i> <i>Staphylococcus aureus</i> <i>Shewanella putrefaciens</i>			<i>Staphylococcus warneri</i> <i>Escherichia coli</i> <i>Kluyveromyces marxianus</i>		
Analysis	Target	F%	Out	Target	F%	Out	Target	F%	Out
Aerob. microorg, 30 °C	<i>H. alvei</i> <i>L. plantarum</i>	1	1	<i>A. hydrophila</i> <i>S. warneri</i> <i>S. aureus</i> <i>S. putrefaciens</i>	0	3	<i>S. warneri</i> <i>E. coli</i>	0	2
Psychrotrophs	<i>H. alvei</i>	8	15	<i>A. hydrophila</i> <i>S. putrefaciens</i>	36	0	<i>S. warneri</i> <i>E. coli</i>	29	0
Enterobacteriaceae	<i>H. alvei</i>	3	2	(<i>A. hydrophila</i>)	23	0	<i>E. coli</i>	1	4
<i>E. coli</i>	-	4	-	-	2	-	<i>E. coli</i>	1	4
Presump. <i>B. cereus</i>	<i>B. cereus</i>	4	5	(<i>A. hydrophila</i>)	14	-	-	4	-
Coag. pos. Staph.	-	2	-	(<i>S. warneri</i>) <i>S. aureus</i>	2	17	(<i>S. warneri</i>)	8	-
Lactic acid bact.	<i>L. plantarum</i>	3	6	-	17	-	-	39	-
<i>C. perfringens</i>	-	1	-	<i>C. perfringens</i>	4	0	-	1	-
Anaerob. sulph. red	-	4	-	<i>C. perfringens</i>	4	3	-	4	-
Aerob. microorg. in fish prod., 20-25 °C	<i>H. alvei</i> <i>L. plantarum</i>	0	0	<i>A. hydrophila</i> <i>S. warneri</i> <i>S. aureus</i> <i>S. putrefaciens</i>	0	6	<i>S. warneri</i> <i>E. coli</i>	3	10
H ₂ S prod. bact. in fish prod.	<i>H. alvei</i>	8	8	<i>S. putrefaciens</i>	4	0	-	0	-
Yeast	-	3	-	-	1	-	<i>K. marxianus</i>	2	4
Moulds	<i>P. verrucosum</i> <i>Aspergillus sp.</i>	3	4	-	1	-	-	4	-

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

Aerobic microorganisms, 30 °C

Mixture A

The colonies counted were mainly from *Lactobacillus plantarum* and *Hafnia alvei* present at the highest concentration in the mixture.

Mixture B

Colonies of *Aeromonas hydrophila*, *Shewanella putrefaciens*, *Staphylococcus warneri* and *Staphylococcus aureus* were counted for this analysis.

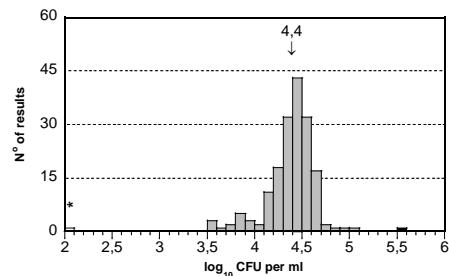
Mixture C

Staphylococcus warneri and *Escherichia coli* were present at the highest concentration in mixture C and therefore formed the colonies counted for the analysis.

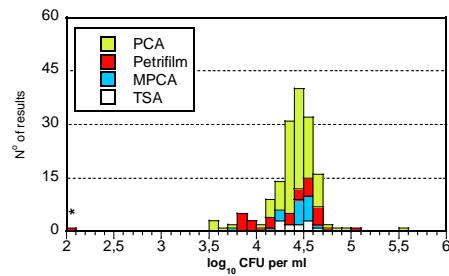
Results of aerobic microorganisms analysis

Medium	Mixture A					Mixture B					Mixture C							
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
Total	176	4.38	0.24	1	0	1	176	4.78	0.15	0	4	2	177	5.06	0.14	0	1	3
PCA	103	4.39	0.23	0	0	1	103	4.75	0.16	0	4	2	104	5.05	0.14	0	1	3
Petrifilm™	31	4.32	0.33	1	0	0	31	4.85	0.10	0	0	0	31	5.08	0.13	0	0	0
MPCA	20	4.40	0.20	0	0	0	20	4.81	0.08	0	0	0	20	5.07	0.10	0	0	0
TSA	11	4.43	0.15	0	0	0	11	4.80	0.12	0	0	0	11	5.06	0.13	0	0	0

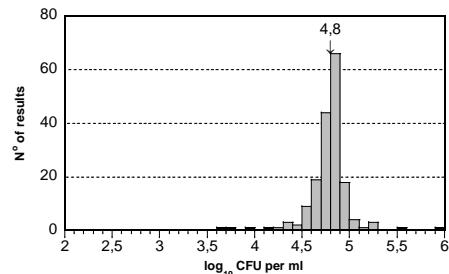
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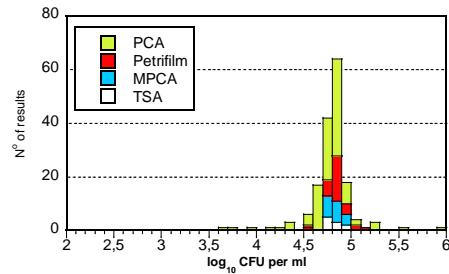
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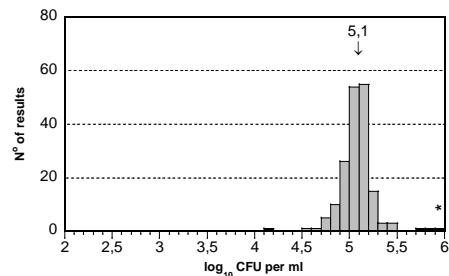
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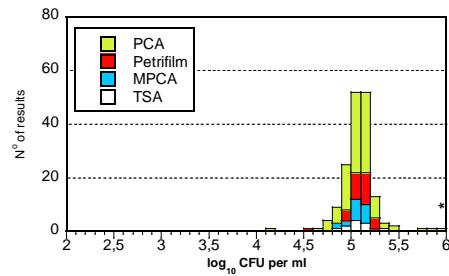
B



C



C



For the three mixtures, there is no obvious difference in results depending on the medium chosen. However, the results for mixture A were globally more spread, with a tail of lower values linked to the use of Petrifilm™.

Psychrotrophic microorganisms

Mixture A

Hafnia alvei was target-organism of the analysis. At NFA, the other strains present in mixture A did not form colonies on PCA after 10 days of incubation at 6.5 °C (NMKL 86:2006).

Mixture B

Aeromonas hydrophila and *Shewanella putrefaciens* can grow at low temperature. However, after 10 days at 6.5 °C colonies were very small and difficult to count without magnifier.

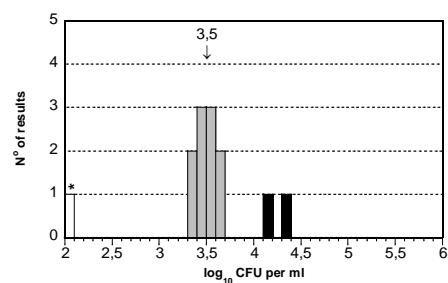
Mixture C

The optimal growth temperature of *Staphylococcus warneri* and *Escherichia coli* present in mixture C is 30-37 °C. At lower temperature these strains grow slower and, at NFA, after 10 days of incubation at 6.5 °C they formed very small colonies difficult to see without magnifier.

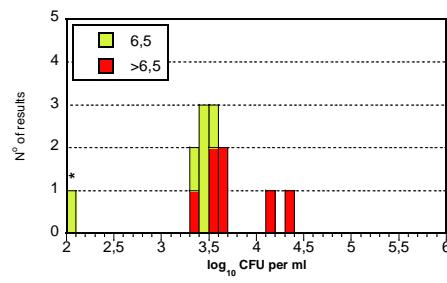
Results of psychrotrophic microorganisms analysis

T °C	Mixture A					Mixture B					Mixture C							
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
Total	13	3.50	0.10	1	0	2	14	3.17	0.71	5	0	0	14	4.11	0.64	4	0	0
6.5	6	3.48	0.08	1	0	0	6	3.17	0.66	3	0	0	6	3.60	0.07	3	0	0
>6.5	7	3.52	0.13	0	0	2	8	3.17	0.80	2	0	0	8	4.34	0.65	1	0	0

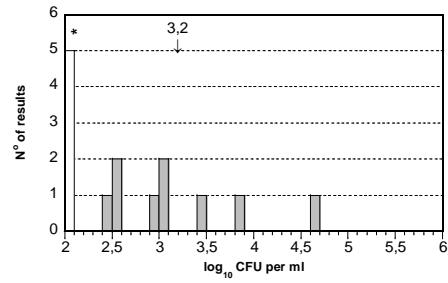
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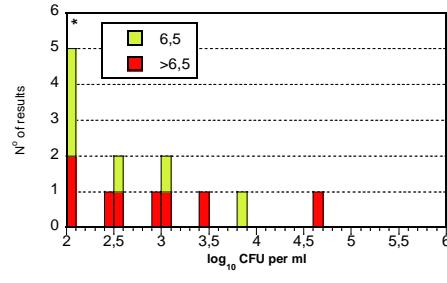
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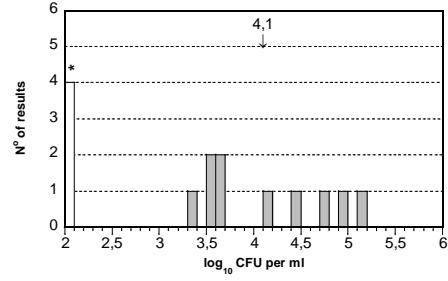
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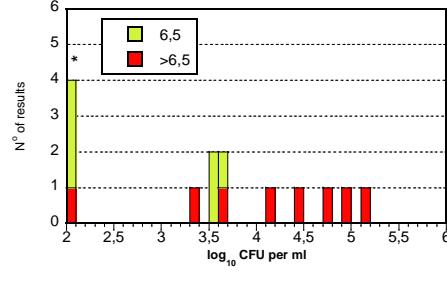
B



C



C



Only 14 laboratories performed this analysis, most of them used PCA as medium but different temperature and time of incubation: 6.5°C / 10 days (NMKL 86:2006), 17°C / 20h + 7°C/ 3 days (NMKL 74:2000), or 21°C / 24h (ISO 8552:2004). This raises the question of the definition of psychrotrophic microorganisms but explains the wide distribution of the results for the three mixtures. Nevertheless, some trends can be seen: at higher temperature of incubation, microorganisms present in the mixture grow faster and might form bigger colonies easier to enumerate. This is reflected by higher values obtained with incubation temperature higher than 6.5°C (mixture C) and the high percentage of false negative results reported by laboratories that incubate plates at 6.5°C during 10 days (mixtures B and C).

Enterobacteriaceae

Mixture A

Hafnia alvei was target-organism for this analysis.

Mixture B

There was no target-organism for this analysis. However, 34 false positive results were reported. *Aeromonas hydrophila* formed red colonies on VRBG but is oxidase-positive and therefore differentiates from enterobacteriaceae.

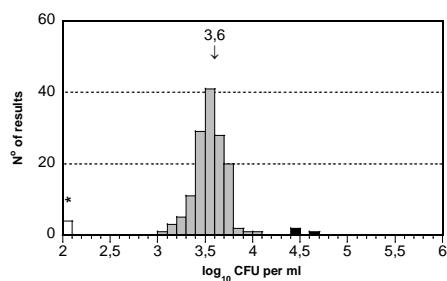
Mixture C

E. coli was target-organism for this analysis.

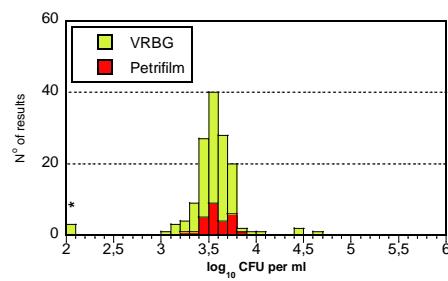
Results of Enterobacteriaceae analysis

Medium	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	149	3.54	0.16	4	0 3	150	- -	-	34	- -	152	4.67	0.23	2	5 1
VRBG	115	3.55	0.16	3	0 3	116	- -	-	18	- -	118	4.65	0.23	2	5 1
Petrifilm™	28	3.44	0.13	1	0 0	28	- -	-	13	- -	28	4.74	0.16	0	0 0

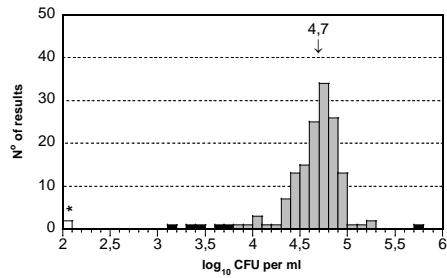
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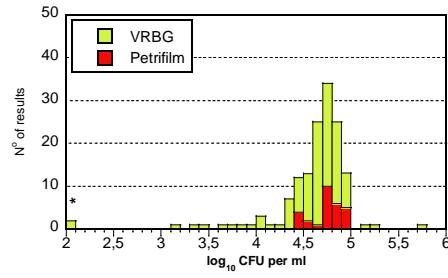
A



C



C



Most of the laboratories used VRBG or Petrifilm™ as medium and similar average results were obtained. For mixture C, many lower values were reported, linked to the use of VRBG. It is possible that the indicator dye present in Petrifilm™ facilitated the reading of colonies for mixture C. On the other hand, half of the laboratories that used Petrifilm™, reported a false positive result for mixture B, indicating that *A. hydrophila* could easily be misjudged as enterobacteriaceae using this medium if no confirmation test was further performed.

Escherichia coli

Mixture A

Even though mixture A did not contain any strain of *Escherichia coli*, six laboratories reported a false positive result. Among them, four used an incubation temperature below 44 °C and did not perform any confirmation.

Mixture B

There was no target-organism for this analysis.

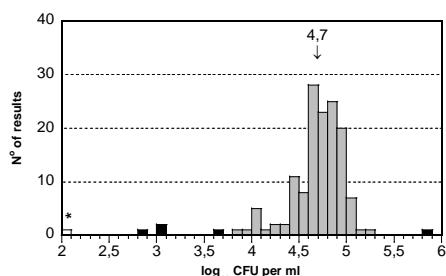
Mixture C

Escherichia coli was target-organism for this analysis.

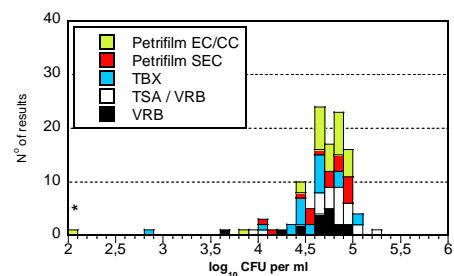
Results of *E. coli* analysis

Medium	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	141	-	-	6	- -	141	-	-	3	- -	142	4.70	0.25	1	4 1
Petrifilm™ EC/CC	29	-	-	2	- -	29	-	-	0	- -	30	4.72	0.21	1	0 0
Petrifilm™ SEC	18	-	-	0	- -	18	-	-	1	- -	18	4.69	0.26	0	0 0
TBX	22	-	-	0	- -	23	-	-	0	- -	23	4.60	0.22	0	1 0
TSA/VRB	24	-	-	1	- -	23	-	-	0	- -	24	4.77	0.29	0	0 0
VRB	17	-	-	2	- -	17	-	-	1	- -	17	4.69	0.20	1	0 0
MPN-based	8	-	-	0	- -	8	-	-	0	- -	8	4.70	0.47	0	1 0

C



C



For mixture C, the results distribution is very similar to the one of enterobacteriaceae analysis where *E. coli* also was target-organism: many low results were reported, but no correlation with the use of a medium can be seen.

Presumptive *Bacillus cereus*

Mixture A

A strain belonging to the *Bacillus cereus* group was target-organism for this analysis.

Mixture B

There was no target-organism for this analysis. On blood-agar, some atypical colonies were surrounded by a haemolytic zone on BcS agar. At NFA, *Aeromonas hydrophila* formed light blue colonies without precipitation zone, while a few laboratories reported the presence of colonies with such zone on their plates. This can explain the report of 19 false positive results.

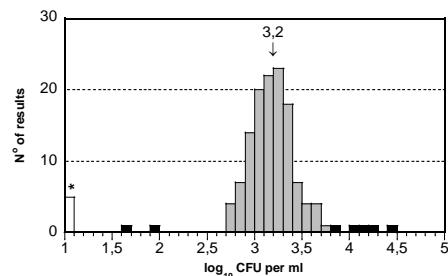
Mixture C

The mixture C did not contain any target-organism for this analysis.

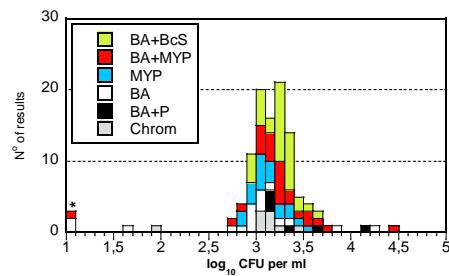
Results of presumptive *B. cereus* analysis

Medium	Mixture A					Mixture B					Mixture C							
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	136	3.17	0.21	5	2	5	136	-	-	19	-	-	136	-	-	5	-	-
BA+BcS	34	3.22	0.17	0	0	0	34	-	-	3	-	-	34	-	-	0	-	-
BA+MYP	25	3.22	0.23	1	0	1	25	-	-	1	-	-	25	-	-	2	-	-
MYP	19	3.12	0.19	0	0	0	19	-	-	1	-	-	19	-	-	1	-	-
BA	17	3.04	0.16	2	1	2	17	-	-	7	-	-	17	-	-	1	-	-
BA+P	6	3.29	0.22	0	0	1	6	-	-	4	-	-	6	-	-	0	-	-
Chrom.	9	3.16	0.15	0	1	0	9	-	-	0	-	-	9	-	-	0	-	-

A



A



No correlation between results and medium can be seen for the analysis of mixture A and C. For mixture B, almost 60% of the false positive results were reported by laboratories that used blood-agar with or without polymyxin as only medium. The NMKL method no 67:2010 describes the confirmation of suspected colonies from blood-agar plates on BcS agar or Cereus-Ident-Agar (chromogenic medium).

Coagulase-positive *Staphylococci*

Mixture A

Mixture A did not contain any coagulase-positive strain of staphylococci.

Mixture B

A strain of *Staphylococcus warneri* and *Staphylococcus aureus* were included in the mixture. Only the latter was target-organism for this analysis. At NFA, colonies of *S. warneri* were atypical, without precipitation zone, on Baird-Parker with rabbit plasma fibrinogen. On BP-agar, they were smaller than those of *S. aureus* and negative when further tested for coagulase activity. Twelve laboratories reported high outliers that can result from the counting of colonies of both strains.

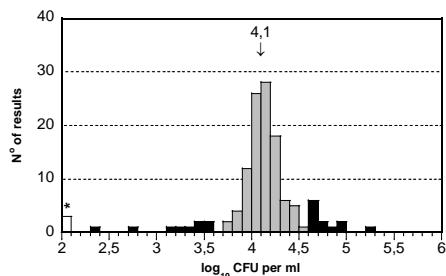
Mixture C

The mixture did not contain any coagulase-positive strain of staphylococci, but a strain of *Staphylococcus warneri*. Ten laboratories reported a false positive result. Among them, five had also reported a result identified as high outlier for mixture B. This suggests that colonies of *S. warneri* were misjudged as coagulase-positive staphylococci in both cases.

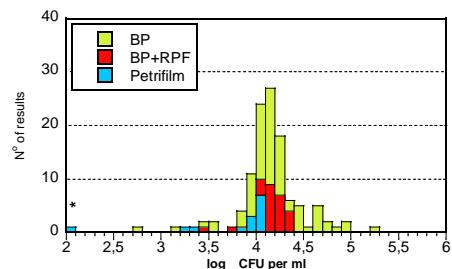
Results of coagulase-positive *Staphylococci* analysis

Medium	Mixture A					Mixture B					Mixture C					
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >	
Total	125	-	-	2	- -	126	4.12	0.16	3	9	12	125	-	-	10	- -
BP	78	-	-	2	- -	79	4.13	0.16	1	5	12	77	-	-	9	- -
BP+RPF	25	-	-	0	- -	25	4.18	0.13	0	1	0	26	-	-	0	- -
Petrifilm™	14	-	-	0	- -	14	4.01	0.06	1	2	0	14	-	-	1	- -

B



B



Almost all high outliers results of mixture B and false positive results of mixture C were 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, almost all laboratories that used BP-agar performed a confirmation step, which suggests that only *S. aureus* colonies were confirmed (mixture B) and/or that the confirmation test failed (mixture B/C). Results of mixture B obtained with Petrifilm™ were slightly lower than the total average. 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, but as in previous rounds, many laboratories reported a false positive result. Both *Staphylococcus warneri* and *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 $1,5 \pm 0,5$ mm after 5 days of incubation at 25°C in anaerobiosis.

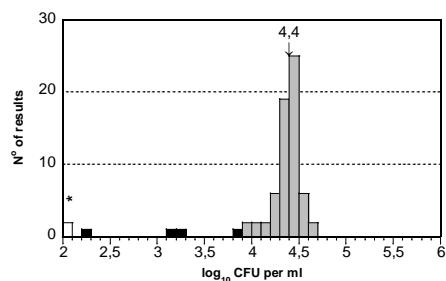
Mixture C

The mixture did not contain any lactic acid bacteria. Almost 40 % of the laboratories reported a false positive result corresponding to the concentration of *Staphylococcus warneri* present in the mixture. *S. warneri* can indeed form small colonies on MRS and even smaller on MRS-aB.

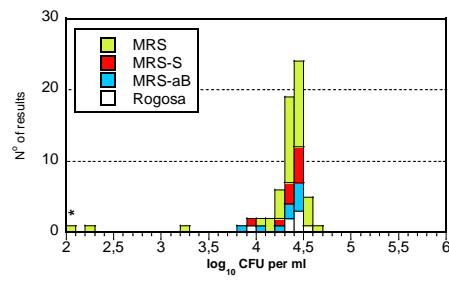
Results of lactic acid bacteria analysis

Medium	Mixture A						Mixture B						Mixture C					
	n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	70	4.38	0.14	2	4	0	69	-	-	12	-	-	69	-	-	27	-	-
MRS	40	4.38	0.13	1	3	0	40	-	-	7	-	-	40	-	-	18	-	-
MRS-S	10	4.35	0.14	0	0	0	9	-	-	1	-	-	9	-	-	1	-	-
MRS-aB	9	4.36	0.14	0	1	0	9	-	-	3	-	-	9	-	-	5	-	-
Rogosa	7	4.37	0.18	0	0	0	7	-	-	0	-	-	7	-	-	2	-	-

A



A



The enumeration of *L. plantarum* in mixture A did not cause any difficulties and all media led to similar results. For the analysis of mixture B and mixture C, one fifth and half of the laboratories using MRS or MRS-aB reported a false positive result, respectively. This suggests that these two media might be less selective than MRS-S and allow the growth of the microorganisms present in the mixtures.

Clostridium perfringens and anaerobic sulphite-reducing bacteria

Mixture A

The mixture did not contain any target-organism for these analyses.

Mixture B

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

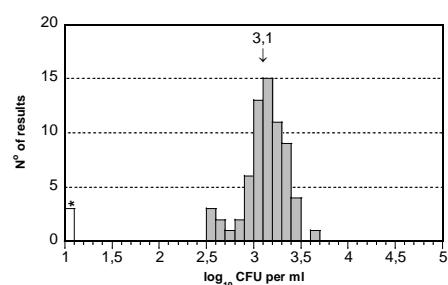
Mixture C

The mixture did not contain any target-organism for these analyses.

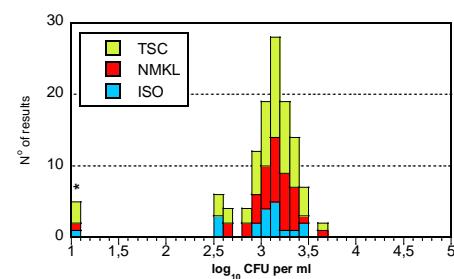
Results of *C. perfringens* analysis

Medium / Method	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	70	-	-	1	- -	70	3.11	0.22	3	0 0	70	-	-	1	- -
TSC	61	-	-	1	- -	91	3.11	0.23	3	0 0	61	-	-	1	- -
NMKL 95:2009	40	-	-	0	- -	40	3.13	0.20	1	0 0	40	-	-	1	- -
EN ISO 7937:2004	19	-	-	0	- -	19	3.04	0.28	1	0 0	19	-	-	0	- -

B



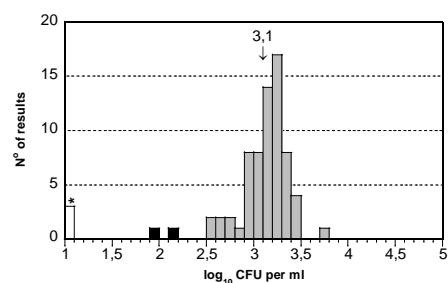
B



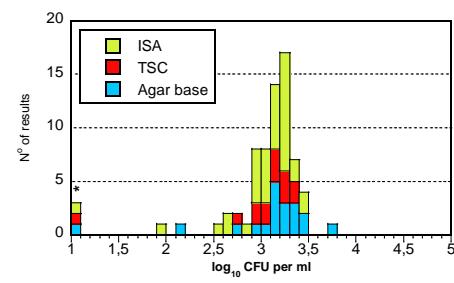
Results of anaerobic sulphite-reducing bacteria analysis

Medium	Mixture A					Mixture B					Mixture C				
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
Total	72	-	-	3	- -	72	3.13	0.22	3	2 0	73	-	-	3	- -
ISA	38	-	-	1	- -	37	3.10	0.20	1	1 0	38	-	-	0	- -
TSC	14	-	-	0	- -	14	3.13	0.16	1	0 0	14	-	-	0	- -
SFP/TSC agar base	19	-	-	2	- -	19	3.22	0.22	1	1 0	19	-	-	3	- -

B



B



These analyses did not cause any difficulties and results for mixture B are approximately the same independently 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. This could be the reason for the slight difference of results between the two methods. For the analysis of anaerobic sulphite-reducing bacteria, slightly higher results were obtained with the use of SFP/TSC agar base. 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.

Aerobic microorganisms, 20-25 °C and H₂S producing bacteria in fish products

Mixture A

Colonies counted for the analysis of aerobic microorganisms were mainly from the strains of *Lactobacillus plantarum* and *Hafnia alvei*. *Hafnia alvei*, which forms black colonies on Iron agar, was also target-organism for the analysis of 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* was target-organism for the analysis of H₂S producing bacteria.

Mixture C

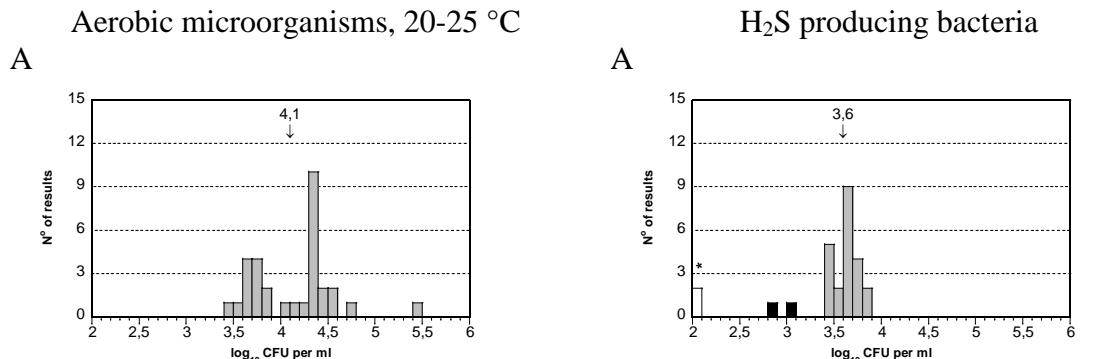
For the analysis of aerobic microorganisms, colonies counted were mainly from the strains of *Staphylococcus warneri* and *Escherichia coli*. The mixture did not contain any H₂S producing bacteria.

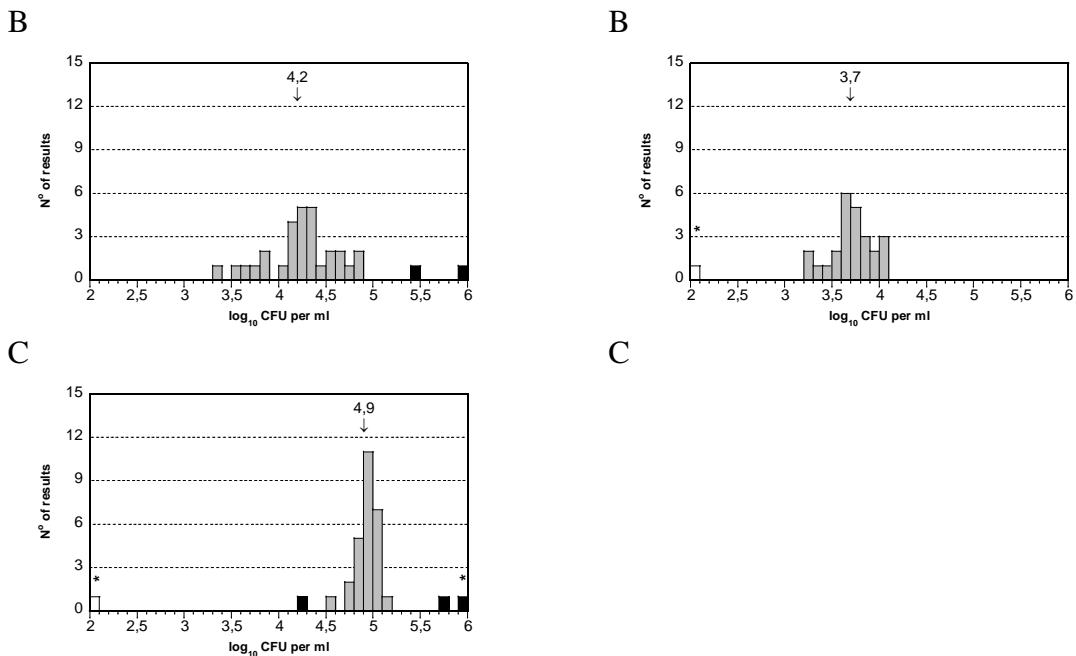
Results of aerobic microorganisms in fish products analysis

Medium	Mixture A					Mixture B					Mixture C							
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
Total	31	4.13	0.43	0	0	0	31	4.23	0.37	0	0	2	31	4.93	0.13	1	1	2
Iron agar	26	4.11	0.45	0	0	0	26	4.21	0.34	0	0	1	26	4.92	0.13	1	0	1

Results of H₂S producing bacteria in fish products analysis

Medium	Mixture A					Mixture B					Mixture C							
	n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
Total	26	3.62	0.13	2	2	0	26	3.70	0.22	1	0	0	26	-	-	0	-	-





The 26 laboratories that performed both analyses all used Iron agar, therefore no distribution of results according to medium is presented here. For the two first mixtures, results of the analysis of aerobic microorganisms are very spread. For mixture A they are divided in two peaks corresponding to the concentration of *H. alvei* (3.6) and *L. plantarum* (4.4). The former corresponds to the peak of results obtained for the analysis of H₂S producing bacteria. The same trend is visible for mixture B, although the results of aerobic microorganisms are distributed in wide peak where the lower values reflect the results obtained for the analysis of H₂S producing bacteria. Mixture C did not cause any difficulty.

Yeasts and moulds

Mixture A

The mixture did not contain any yeast. Strains of *Penicillium verrucosum* and *Aspergillus sp.* were target-organisms for the analysis of moulds. *P. verrucosum* formed small colonies on DRBC and brick-colored colonies on DG18. *Aspergillus sp.* formed blue-green colonies both on DRBC and DG18.

Mixture B

The mixture contained no yeasts or moulds.

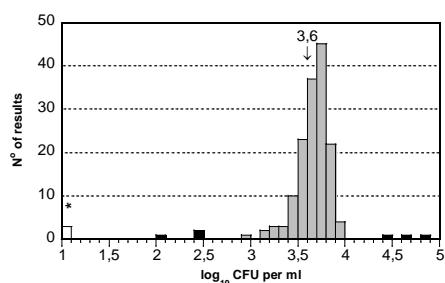
Mixture C

The mixture contained no moulds but a yeast strain of *Kluyveromyces marxianus* which formed pink colonies on DRBC and small white colonies on DG18.

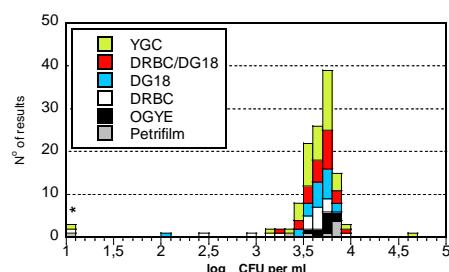
Results of yeast analysis

Medium	Mixture A				Mixture B				Mixture C			
	n	m	s	F	<	>	n	m	s	F	<	>
Total	154	-	-	5	-	-	157	-	-	2	-	-
YGC	36	-	-	1	-	-	44	-	-	0	-	-
DRBC/DG18	17	-	-	0	-	-	25	-	-	0	-	-
DG18	13	-	-	1	-	-	20	-	-	0	-	-
DRBC	15	-	-	1	-	-	17	-	-	1	-	-
OGYE	8	-	-	0	-	-	10	-	-	0	-	-
Petrifilm™	5	-	-	0	-	-	8	-	-	0	-	-

C



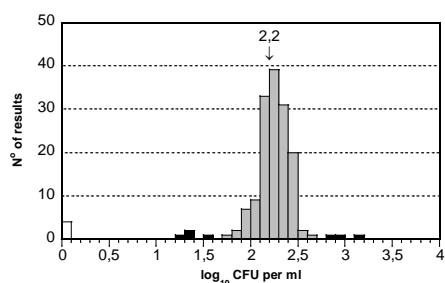
C



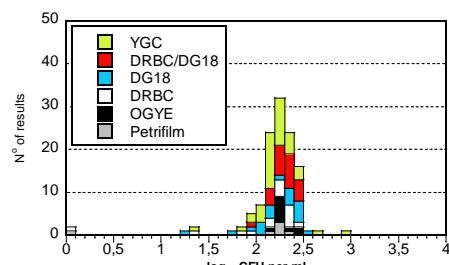
Results of mould analysis

Medium	Mixture A				Mixture B				Mixture C			
	n	m	s	F	<	>	n	m	s	F	<	>
Total	156	2.24	0.15	4	4	3	157	-	-	2	-	-
YGC	42	2.21	0.15	0	1	1	42	-	-	2	-	-
DRBC/DG18	25	2.28	0.12	0	0	0	25	-	-	0	-	-
DG18	19	2.22	0.21	0	1	0	20	-	-	0	-	-
DRBC	16	2.28	0.15	1	1	0	16	-	-	0	-	-
OGYE	10	2.28	0.12	0	0	0	10	-	-	0	-	-
Petrifilm™	5	2.21	0.07	1	0	0	7	-	-	0	-	-

A



A



Most of the laboratories performed yeast and mould analyses according to the method NMKL 98:2005 / 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 describe the use of YGC or OGYE. Few laboratories used the method ISO 7954:1987 that has been replaced by ISO 21527. There is no obvious difference in results depending on the medium used.

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.

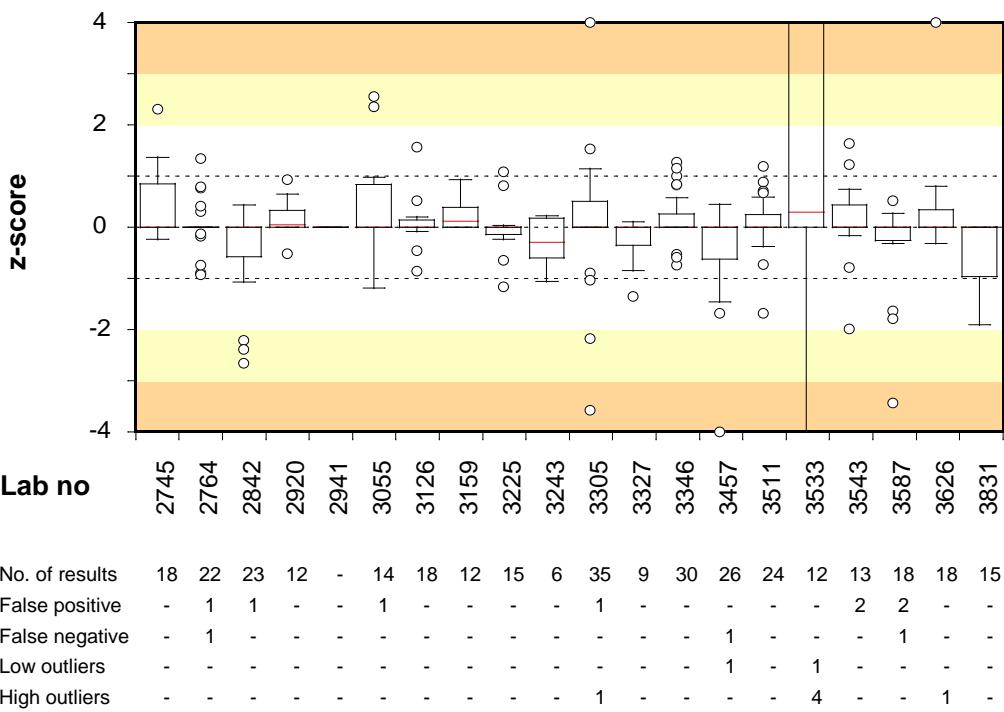
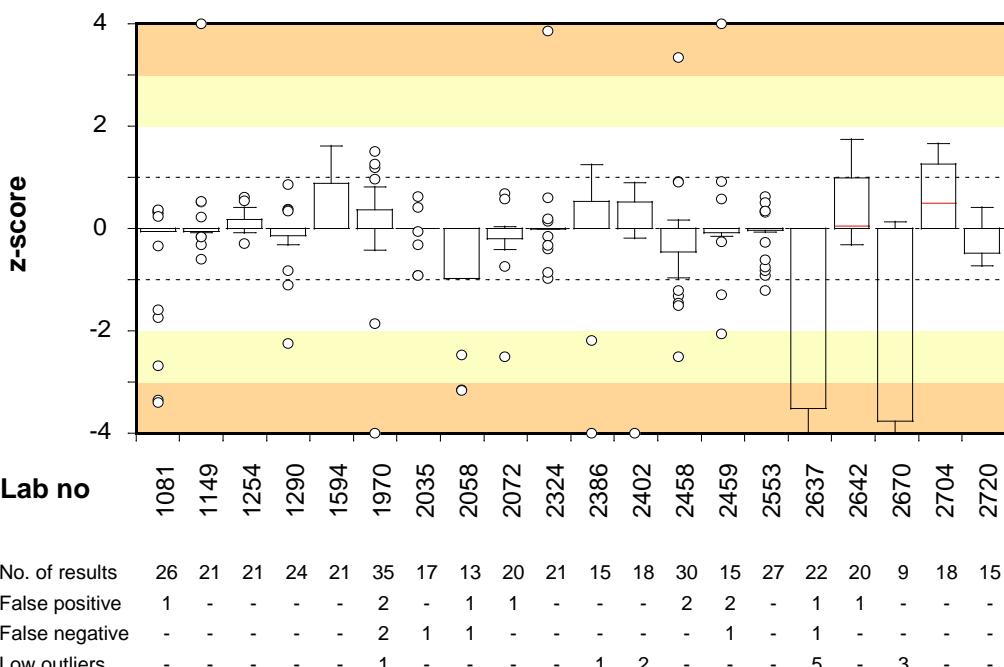
All the results from each laboratory – outliers included and false results excluded – were compiled into a box plot based on their z-scores. The smaller and more centred round zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

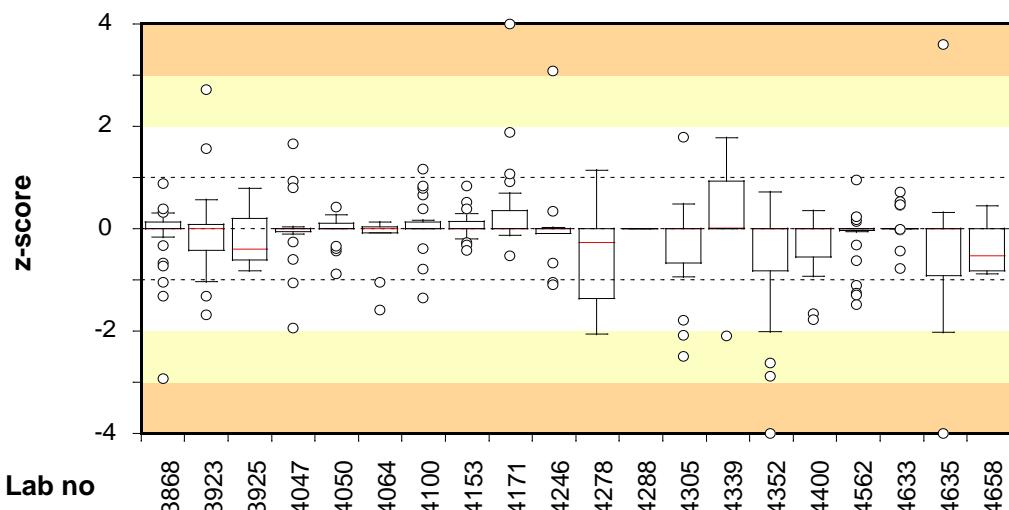
The laboratories were not grouped or ranked based on their results. However, for each laboratory, the numbers of false results and outliers are presented below the box plots. These results are also highlighted in Annex 1, where all the reported results are listed, and the minimum and maximum accepted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

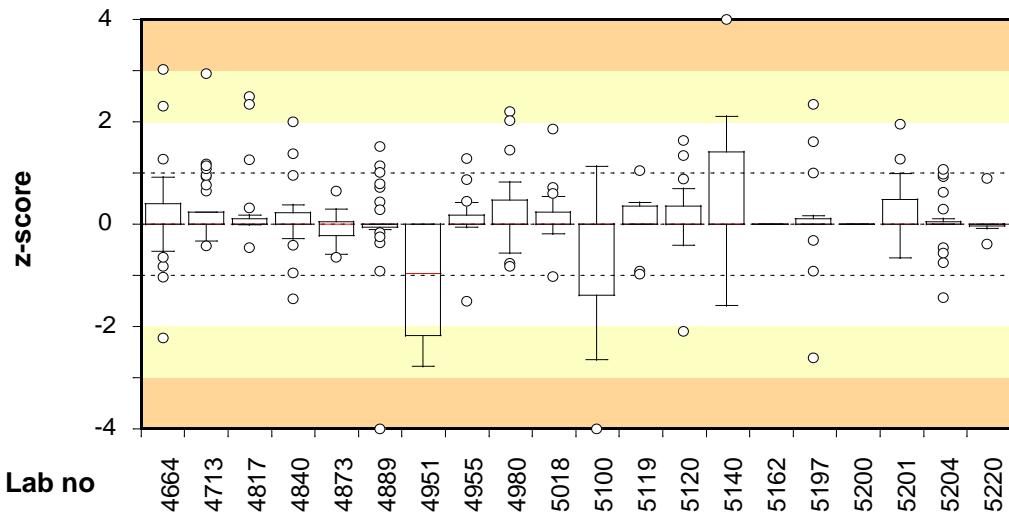
Box plots and numbers of deviating results for each laboratory

- *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 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 – $1.5 \times (\text{the highest result in the box} - \text{the lowest result in the box})$ or the highest result in the box + $1.5 \times (\text{the highest result in the box} - \text{the lowest result in the box})$. 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.*

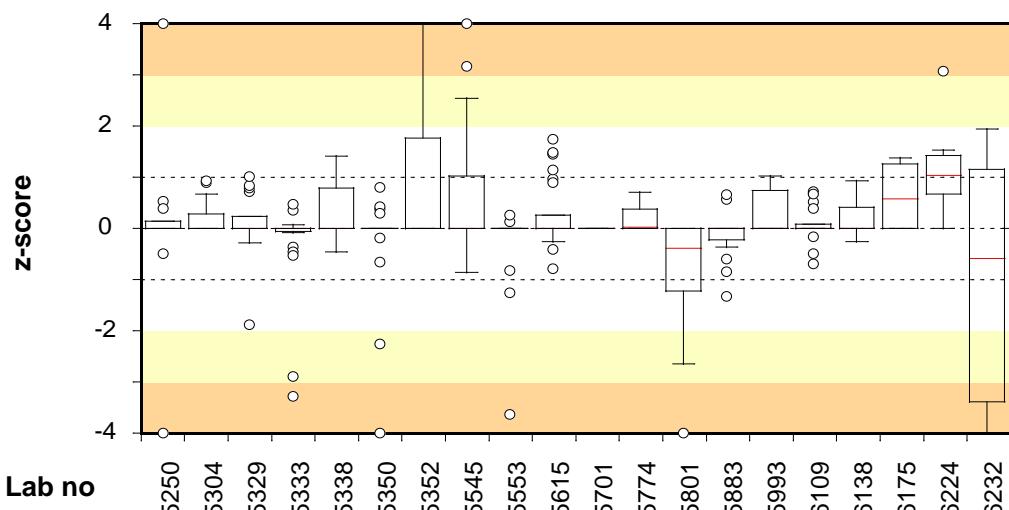




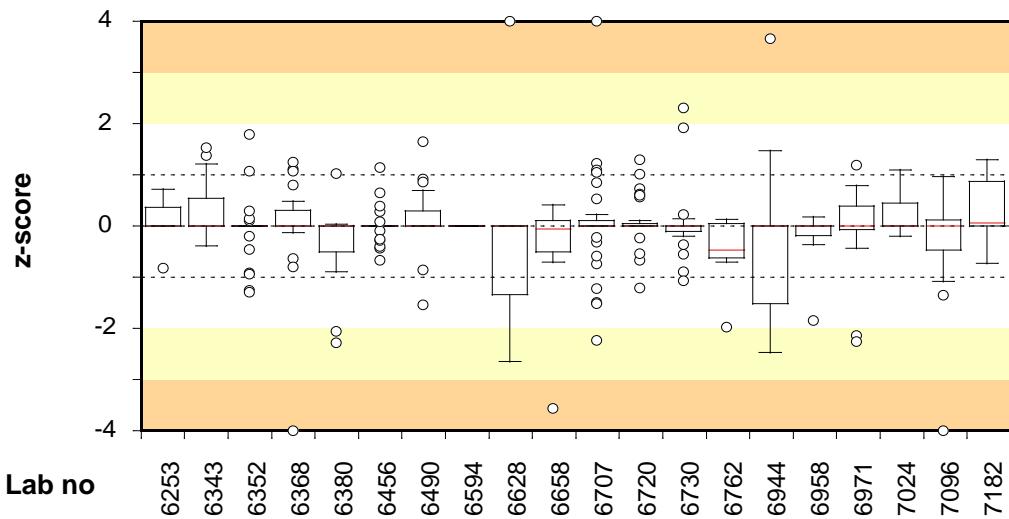
Lab no	3868	3923	3925	4047	4050	4064	4100	4153	4171	4246	4278	4288	4305	4339	4352	4400	4562	4633	4635	4658
No. of results	36	20	3	20	17	9	29	33	24	13	14	-	22	34	22	14	27	18	22	5
False positive	-	4	-	-	1	-	1	-	-	2	1	-	2	2	2	1	-	-	2	2
False negative	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-
High outliers	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	1	-



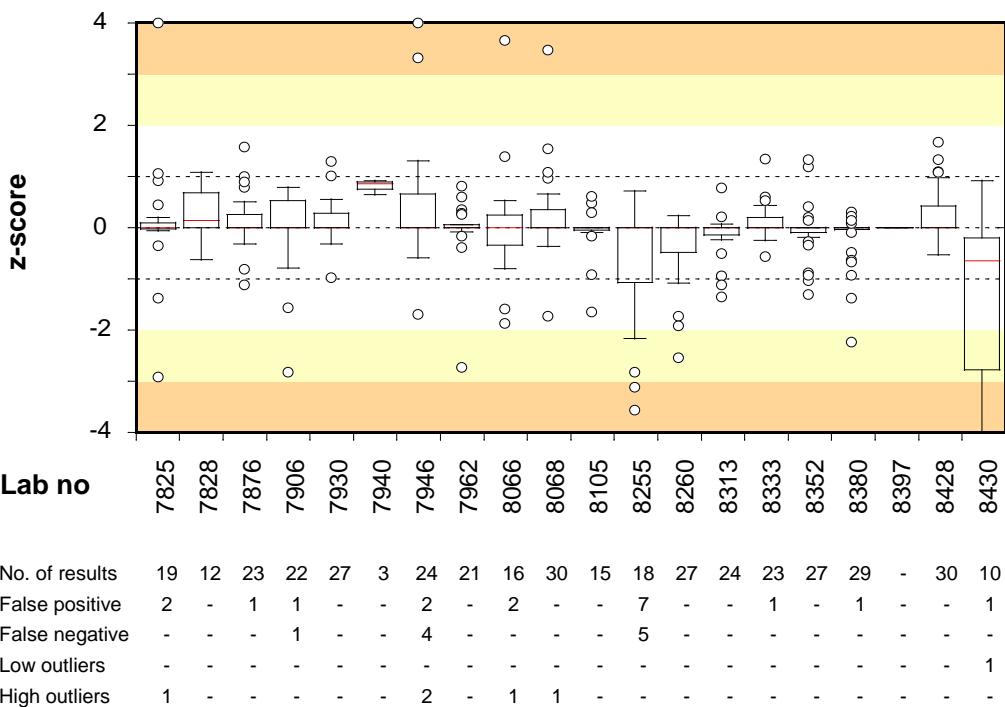
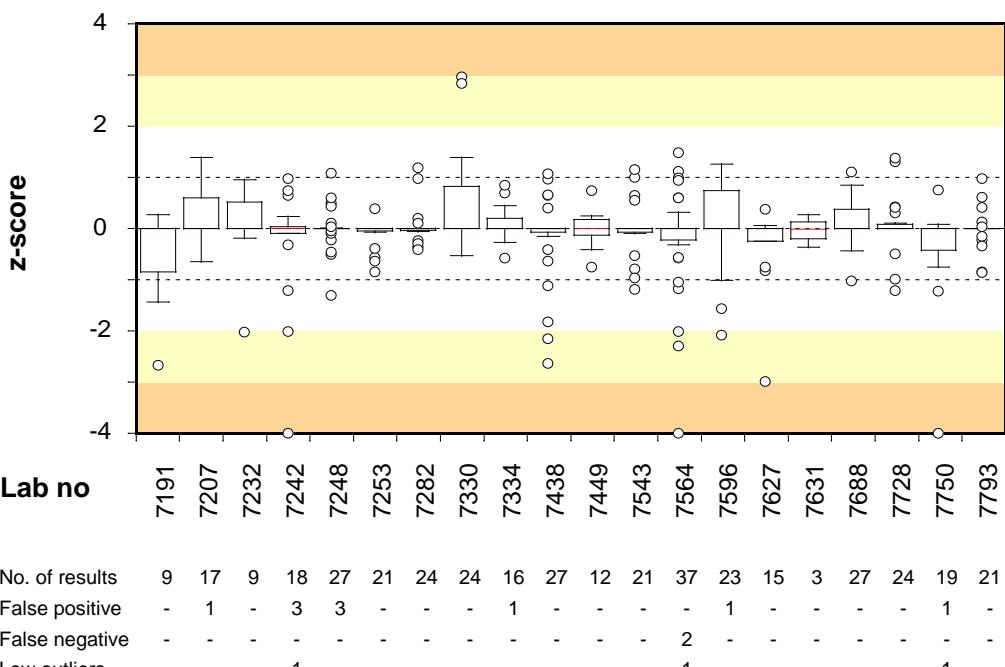
Lab no	4664	4713	4817	4840	4873	4889	4951	4955	4980	5018	5100	5119	5120	5140	5162	5197	5200	5201	5204	5220
No. of results	23	34	21	21	12	30	12	30	21	28	12	9	30	22	-	17	-	21	24	12
False positive	1	-	-	3	-	-	3	-	-	1	-	-	2	-	2	-	-	2	-	-
False negative	-	2	-	-	-	-	-	-	-	1	-	-	-	-	2	-	-	1	-	-
Low outliers	-	-	-	-	-	2	-	-	-	-	2	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-

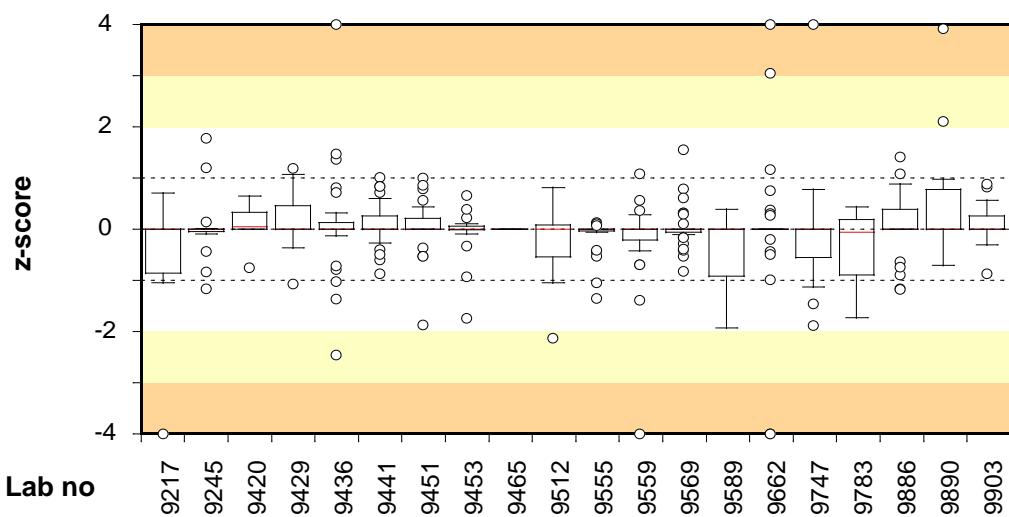
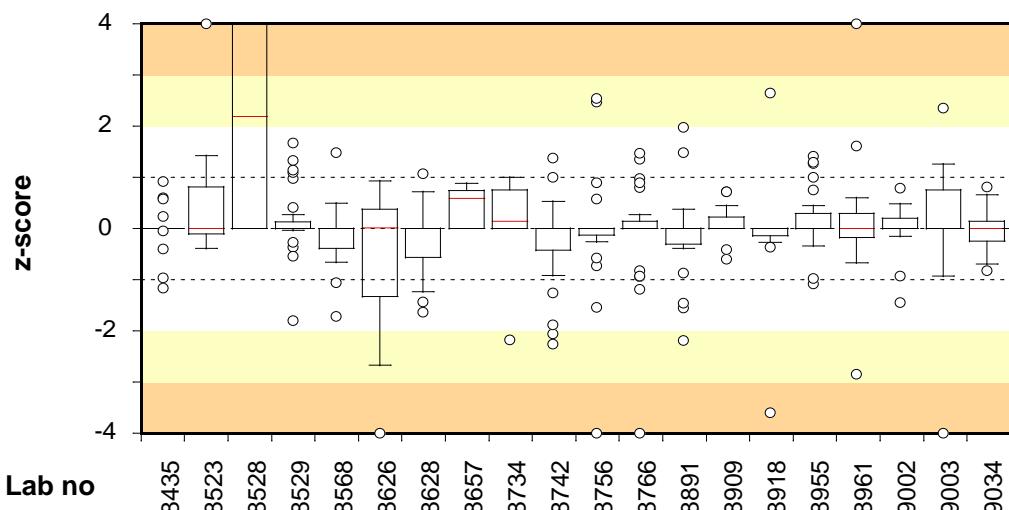


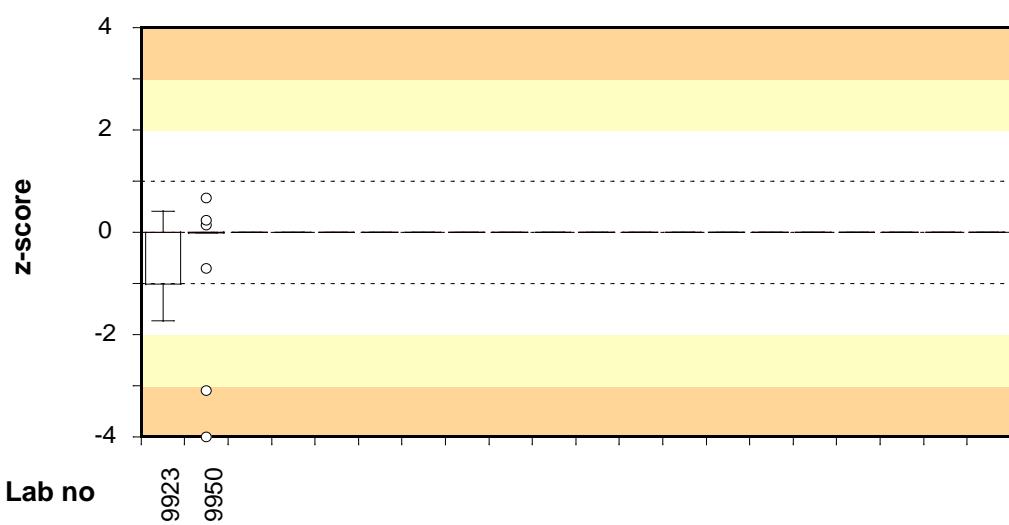
	5250	5304	5329	5333	5338	5350	5352	5545	5553	5615	5701	5774	5801	5883	5993	6109	6138	6175	6224	6232
No. of results	14	15	21	23	9	17	23	19	13	29	-	4	13	24	6	21	24	4	7	6
False positive	4	-	-	-	-	1	1	-	-	1	-	-	-	2	-	-	-	-	2	-
False negative	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	2	-	-	-	-	1	-	-	1	-	-	-	-	1	-	-	-	-	-	1
High outliers	1	-	-	-	-	-	1	2	-	-	-	-	-	-	-	-	-	-	1	-



	6253	6343	6352	6368	6380	6456	6490	6594	6628	6658	6707	6720	6730	6762	6944	6958	6971	7024	7096	7182
No. of results	23	24	25	33	12	27	21	-	9	8	33	27	19	7	24	8	13	15	15	16
False positive	1	-	1	-	-	-	-	-	-	1	2	-	2	2	-	1	2	-	-	2
False negative	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-
Low outliers	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
High outliers	-	-	-	-	-	-	-	-	1	-	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>Hafnia alvei</i>	SLV-015
	<i>Bacillus cereus</i>	SLV-202
	<i>Lactobacillus plantarum</i>	SLV-445
	<i>Penicillium verrucosum</i>	SLV-544
	<i>Aspergillus sp.</i>	SLV-484
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>Staphylococcus warneri</i>	SLV-565
	<i>Escherichia coli</i>	SLV-082
	<i>Kluyveromyces marxianus</i>	SLV-439

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 was performed in conjunction with manufacturing of the mixtures according to Scheme Protocol (3). The results are presented in Table 3. Homogeneity requires that the standard deviation and the difference between the highest and lowest value of results from 10 samples analysed do not exceed 0.15 log₁₀ units and 0.5 log₁₀ units, respectively.

Table 3. Concentration mean (*m*) and standard deviation (*s*) from analyses of 10 randomly selected vials per mixture, expressed in log₁₀ 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	4.61	0.07	4.83	0.04	5.12	0.03
Psychrotrophic microorganisms NMKL method no. 83	3.78	0.09	3.18	0.14	3.91	0.08
Enterobacteriaceae NMKL method no. 144	3.88	0.08	—	—	4.81	0.06
<i>Escherichia coli</i> NMKL method no. 125	—	—	—	—	4.83	0.05
Presumptive <i>Bacillus cereus</i> NMKL method no. 67	3.31	0.08	—	—	—	—
Coagulase-positive staphylococci NMKL method no. 66	—	—	4.20	0.03	—	—
Lactic acid bacteria NMKL method no. 140	4.48	0.09	—	—	—	—
<i>Clostridium perfringens</i> NMKL method no. 95	—	—	3.03	0.05	—	—
Anaerobic sulphite-reducing bacteria NMKL method no. 56	—	—	3.26	0.05	—	—
Aerobic microorganisms in fish products NMKL method no. 184, IA	4.60	0.09	4.72	0.03	5.07	0.05
H ₂ S-producing bacteria in fish products NMKL method no 184, IA	3.68	0.15	4.04	0.05	—	—
Yeasts NMKL method no. 98, DRBC	—	—	—	—	3.78	0.05
Moulds NMKL method no. 98, DRBC	2.33	0.07	—	—	—	—

— No target organism

References

1. Kelly K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58-64.
2. Harmoni SM, Kautter DA, Peeler JT. 1971. Improved medium for enumeration of *Clostridium perfringens*. *Appl. Microbiol.* 22:688-92.
3. Anonymous, 2012. Protocol. *Microbiology. Drinking Water & Food*. The National Food Agency.
4. Peterz M, Steneryd AC. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *J. Appl. Bacteriol.* 74:143-148.

Annex 1 Results of the participating laboratories - April 2013

All results are expressed in \log_{10} cfu per ml sample.

Results reported as " $<$ value" have been regarded as zero (negative).

Results reported as " $>$ value" are excluded of the calculations.

A dash in the table indicates that the analysis was not performed.

Outliers and false results are highlighted and summarized for each analysis at the end of the table.

Lab no.	Vial	Aerobic microorganisms 30°C			Enterobacteriaceae			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive staphylococci			Lactic acid bacteria			Clostridium perfringens			Anaerobic sulphite-reducing bacteria			Psychrotrophic microorganisms			Aerobic m.o. in fish products, 20-25 °C			H_2S producing bacteria			Yeast			Lab no				
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C					
1081	3 1 2	4.45	4.83	5.11	3.49	2	3.91	<1	<1	3.86	2.81	<2	<2	<1	4.18	<1	-	-	-	<1	2.5	<1	<1	2.79	<1	-	-	-	-	-	-	<1	<1	3.69	2.23	<1	<1	1081				
1149	3 1 2	4.3	4.86	5.04	3.45	<2	4.72	<2	<2	4.66	4.18	<1	<1	<2	4.11	<2	-	-	-	<1	3.2	<1	-	-	-	-	-	-	<1	<1	3.74	2.23	<1	<1	1149							
1254	2 3 1	-	-	-	-	-	-	<2	<2	4.74	-	-	-	<2	4.15	<2	-	-	<1	3.2	<1	-	-	-	-	-	-	0	0	3.72	2.32	0	0	1254								
1290	1 3 2	4.33	4.84	4.95	3.37	<1	4.65	<1	<1	4.65	3.35	<1	<1	<1	4.18	<1	-	-	-	<1	3	<1	-	-	-	-	-	-	<1	<1	3.28	2.29	<1	<1	1290							
1594	1 3 2	4.61	4.88	5.23	3.74	<1	4.87	<2	<2	4.98	3.28	<2	<2	<2	4.15	<2	-	-	-	<1	3	<1	-	-	-	-	-	-	0	0	3.76	2.48	0	0	1594							
1970	2 3 1	4.32	4.78	5.16	3.58	<2	4.73	<2	<2	4.79	3.34	<1	<1	<2	4.18	<2	4.51	<2	<2	<1	3.4	<1	1.78	3.15	3.08	3.31	<1	<1	4.22	4.7	5.1	2.85	3.61	<2	<1	<1	3.9	2.3	<1	<1	1970	
2035	1 3 2	-	-	-	3.4	<2	4.6	-	-	-	3.3	<1	-	<2	<2	<2	<1	3.1	<1	-	-	-	-	-	-	-	<1	<1	3.5	2.3	<1	<1	2035									
2058	1 2 3	4.14	4.32	4.63	-	-	-	<1	<1	4.09	<2	<2	4.23	<1	4	<1	-	-	<1	3	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2058				
2072	3 1 2	4.54	4.87	5.04	3.48	2.29	4.64	<1	<1	4.64	3.18	<1	<1	-	-	-	-	-	<1	2.6	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2072						
2324	3 2 1	4.41	4.64	5.06	3.52	0	4.58	0	0	4.62	3.21	0	0	0	4.72	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2324						
2386	2 3 1	3.85	4.94	5.18	-	-	-	<2	<2	4.81	3.3	<1	<1	<2	3.3	<2	-	-	-	-	-	-	<1	3.4	<1	-	-	-	-	-	-	-	-	-	-	-	-	2386				
2402	3 2 1	4.59	4.86	5.16	3.52	<1	4.83	<1	<1	4.87	-	-	-	-	-	-	-	-	-	<1	1.9	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	2402						
2458	3 1 2	4.39	4.59	4.93	3.69	<1	4.34	<1	<1	4.08	2.92	<1	<1	<1	4.64	<1	4.17	4.4	4.81	<1	3.2	<1	<1	3.15	<1	-	2.93	4.7	-	-	-	-	-	<1	<1	3.54	2.17	<1	<1	2458		
2459	2 1 3	3.88	4.87	5.04	-	-	-	0	0	4.93	2.9	0	0	0	4.08	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2459					
2553	2 1 3	4.45	4.65	4.95	3.6	<2	4.5	<2	<2	4.4	3.3	<1	<1	<2	4.2	<2	-	-	-	<1	3.1	<1	<1	3	<1	-	-	-	-	-	<1	<1	3.64	2.29	<1	<1	2553					
2637	2 1 3	3.53	3.76	4.18	<1	<1	3.45	<1	<1	4	2.92	<1	<1	<1	3.15	<1	-	-	-	<1	2.17	2.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2637					
2642	1 3 2	4.5	4.8	5.2	3.7	3.1	4.9	<2	<2	5	-	-	-	<2	4.1	<2	-	-	-	-	-	-	-	3.6	3.4	5.1	-	-	-	-	-	<1	<1	3.6	2.5	<1	<1	2642				
2670	1 2 3	4.17	4.17	5.08	-	-	-	0	0	3.04	-	-	-	<0	3.54	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2670					
2704	1 3 2	4.56	4.97	5.12	3.79	<2	4.81	<2	<2	4.84	-	-	-	<2	4.38	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2704						
2720	3 2 1	4.26	4.81	5.11	3.43	<1	4.55	-	-	-	3.05	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2720						
2745	3 2 1	4.58	4.88	5.03	3.68	<2	4.98	<2	<2	4.9	3.36	<1	<1	<2	4.2	<2	-	-	-	<1	3.6	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	2745						
2764	3 2 1	4.56	4.83	4.94	3.67	2.8	4.63	<0.60	<0.60	4.47	3.45	<1	<1	-	-	-	4.36	<2	<2	-	-	<1	<1	<1	-	-	-	-	-	-	-	-	-	-	-	2764						
2842	1 3 2	-	-	-	3.57	2.99	4.77	-	-	-	2.96	0	0	0	3.78	0	4.23	0	0	0	2.9	0	0	2.56	0	-	-	-	-	-	0	0	3.61	1.88	0	0	2842					
2920	1 3 2	4.44	4.8	5.19	3.57	0	4.76	0	0	4.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2920					
2941	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2941
3055	2 1 3	4.61	4.74	4.9	3.95	0	4.83	-	-	-	3.66	3.1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3055				
3126	1 2 3	4.5	4.86	5.09	-	-	-	<2	<2	4.68	3.2	<1	<1	<2	3.99	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3126					
3159	2 1 3	4.6	4.84	5.07	3.59	<2	4.76	<2	<2	4.87	-	-	-	<2	4.15	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3159					
3225	2 3 1	4.22	4.69	5.03	3.36	<1	4.66	-	-	-	3.18	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3225				
3243	2 1 3	4.12	4.7	4.98	3.58	<2	4.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3243			
3305	2 1 3	4.56	4.9	5.15	3.2	<2	3.86	2	<2	5.08	3.2	<1	<1	<2	4.3	<2	4.45	<2	4.98	<1	3.3	<1	<1	3.2	<1	-	-	-	4.36	4.3	5.7	3.51	3.81	<2	0	0	3.48	2.3	0	0	3305	
3327	1 3 2	-	-	-	3.33	<1	4.59	<1	<1	4.49	-	-	-	<1	4.14	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3327				
3346	2 1 3	4.43	4.87	4.99	3.61	<1	4.73	<1	<1	4.76	3.38	<2	<2	<2	4.32	<2	4.49	<2	<2	<1	3.3	<1	<1	3.38	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3346		
3457	2 1 3	4.48	4.76	5.23	3.23	1.77	5.04	-	-	-	3.2	<1	<1	<2	4	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3457					
3587	2 1 3	4.5	4.8	5.1	3	<2	4.3	3	<2	4.7	2.8	<1	<1	-	-	-	-	-	-	-	-	-	-	<1	<1	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	3587		
3626	1 3 2	4.3	4.8	5.1	3.6	<2	4.8	<2	<2	4.9	4.2	<1	<1	<2	4.2	<2	-	-	-	<1	3.1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3626					
3831	3 2 1	4.25	4.58	4.87																																						

Lab no.	Vial	Aerobic microorganisms 30°C			Enterobacteriaceae			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive staphylococci			Lactic acid bacteria			Clostridium perfringens			Anaerobic sulphite-reducing bacteria			Psychrotrophic microorganisms			Aerobic m.o. in fish products, 20-25 °C			H ₂ S producing bacteria			Yeast			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								
8657	3 2 1	4.54	4.87	5.15	3.64	<1	4.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	3.79	2.36	<1	<1	8657								
8734	2 1 3	4.6	4.8	5.2	3.2	0	4.8	-	-	-	-	-	-	-	-	-	4.4	0	3.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8734							
8742	3 1 2	4.3	4.6	4.78	3.4	<1	4.6	<1	<1	5.04	3.38	<1	<1	<1	4.04	<1	-	-	-	<1	3.2	<1	-	-	-	-	-	-	-	<1	<1	3.34	1.9	<1	<1	8742						
8756	2 3 1	4.2	4.7	5.4	3.3	2.8	4.8	<2	<2	4.7	3.7	<1	1	<2	2.3	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8756									
8766	2 1 3	4.7	5	5.1	3.7	<1,3	4.4	<2	<2	4.9	3	<1	<1	<2	2.7	<2	-	-	-	<1	2.9	<1	-	-	-	-	-	-	-	<1	<1	3.8	2.2	<1	<1	8766						
8891	3 2 1	4.85	4.84	4.85	3.78	<1	4.64	<1	<1	4.16	2.99	3.2	<1	<1	4.09	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	3.41	2.18	<1	3.39	8891						
8909	3 1 2	4.23	4.85	5.08	3.66	<2	4.72	<2	<2	4.6	3.32	<2	<2	<2	4.18	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	3.7	2.24	<1	<1	8909						
8918	3 2 1	5.01	4.73	4.57	-	-	-	4.8	<2	<2	<1	<1	3.15	<2	4.08	<2	<2	4.35	-	-	-	-	-	-	-	-	-	-	-	3.57	0	0	0	0	2.28	8918						
8955	1 2 3	4.38	4.85	5.2	3.49	<2	4.68	<2	<2	4.95	3.26	<1	<1	<2	4.32	<2	<2	<1	3.4	<1	<1	3.41	<1	-	-	-	3.67	3.9	4.9	3.67	3.87	<2	<1	<1	3.69	2.2	<1	<1	8955			
8961	1 2 3	3.69	4.78	4.97	4.43	2.3	4.6	-	-	-	0	3.48	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3.75	2.48	0	0	8961							
9002	2 1 3	4.49	4.8	5.11	3.52	<2	4.72	<2	<2	4.78	2.87	<1	<1	<2	4.15	<2	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3.7	2.1	0	0	9002							
9003	3 2 1	4.53	5.13	5.18	3.55	3.22	4.46	<1	<1	4.69	-	-	-	<1	3.22	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9003					
9034	3 2 1	4.57	4.87	4.99	3.5	<2	4.82	<2	<2	4.77	-	-	-	-	-	-	4.36	<2	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9034					
9217	3 1 2	4.28	4.63	5.1	3.59	<2	4.83	-	-	-	2.97	3.18	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	2.46	2.11	0.7	<1	9217				
9245	1 2 3	4.41	4.72	5.05	3.36	<2	4.48	-	-	-	3.54	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9245						
9420	1 2 3	4.4	4.85	5.11	3.57	<1	4.74	<1	<1	4.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9420							
9429	2 1 3	4.6	4.9	5.18	3.54	<1	4.64	<1	<1	4.61	2.95	<1	<1	<1	4.23	<1	-	-	-	<1	3.4	<1	<1	3.2	<1	-	-	-	-	-	-	-	-	-	-	-	9429					
9436	2 1 3	4.57	4.89	5.87	3.57	<1	4.36	<1	<1	4.74	3.19	<1	<1	<1	4	<1	4.04	4.7	4.45	<1	3	<1	<1	3.2	<1	3.64	2.44	4.18	-	-	-	-	-	-	-	<1	<1	3.63	2.46	<1	<1	9436
9441	3 1 2	4.46	4.85	5.11	3.45	<2	4.58	<2	<2	4.88	3.15	<1	<1	<2	4.28	<2	4.46	<2	<2	<1	3.3	<1	<1	3.23	<1	3.45	3.04	3.56	4.3	4.3	4.9	3.59	3.74	<2	<1	<1	3.79	2.23	<1	<1	9441	
9451	2 3 1	4.41	4.73	5.08	3.46	<1	4.77	<1	<1	4.74	3.38	<1	<1	<1	4.04	<1	-	-	-	<1	3.2	<1	<1	3.3	<1	-	-	-	-	-	-	-	-	-	-	-	-	9451				
9453	1 3 2	4.43	4.77	5.06	3.58	<1	4.46	-	-	-	2.81	<1	<1	<1	4.14	<1	4.43	4.6	5.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9453				
9465	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9465					
9512	2 1 3	4.49	4.81	4.92	3.48	2.63	4.52	-	-	-	3.34	2.9	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	<1	3.3	2.18	<1	<1	9512				
9555	1 2 3	4.37	4.8	5.08	3.33	<1	4.57	<1	<1	4.57	3.16	<1	<1	<1	3.96	<1	-	-	-	<1	3	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9555				
9559	3 1 2	4.21	4.76	5.14	3.59	2.6	4.61	<1	<1	4.53	3.14	3	<1	<1	4.18	<1	3.11	<1	3.74	-	-	-	<1	3.04	<1	-	-	-	-	-	-	-	-	-	-	-	-	9559				
9569	3 2 1	4.35	4.8	5.04	3.5	<2	4.85	<2	<2	4.78	3	<1	<1	<2	4.04	<2	4.32	<2	3.77	<1	3.1	<1	<1	3.2	<1	-	-	-	4.4	4.8	4.9	-	-	-	-	-	-	9569				
9589	3 1 2	4.32	4.73	4.99	3.57	2.32	4.43	<1	<1	4.43	2.95	<1	<1	<1	3.98	<1	4.43	<2	<2	<1	2.7	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9589				
9662	3 1 2	5.52	4.84	5.48	3.73	<1	4.74	<2	<2	4.65	3.08	3	<1	<2	3.45	<2	4.48	<2	<2	<1	3	<1	<1	2.92	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	9662			
9747	3 2 1	4.3	4.73	4.96	4.45	<1	4.34	-	-	-	2.78	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747						
9783	2 1 3	3.96	4.85	5.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9783					
9886	3 1 2	4.52	4.89	5.21	3.36	<1	4.85	<2	<3	4.87	3.04	<1	<1	<2	4.24	<2	4.43	<2	<2	<1	3.2	<1	<1	3.32	<1	3.53	2.53	3.36	-	-	-	-	-	-	-	-	-	-	9886			
9890	1 2 3	4.61	4.68	5.1	3.88	3.05	4.87	0	0	4.9	3.07	0	0	0	4.73	4.55	4.43	0	3.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9890				
9903	3 1 2	4.31	4.83	5.02	3.62	0	4.72	0	0	4.84	3.21	0	0	0	4.25	0	-	-	-	0	3.2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9903					
9923	3 1 2	4.23	4.53	4.83	3.31	<2	4.44	<2	<2	4.44	-	-	-	<2	4.01	<2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9923					
9950	3 1 2	4.41	4.33	5.06	-	-	-	-	-	-	1.6	<1	<1	-	-	-	4.28	4.3	4.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950		

n = number of analyses performed
Min = lowest reported result
Max= highest reported result
Median = median value
m = mean value
s = standard deviation

F+ = false positive
F- = false negative
< = low outlier
> = high outlier
< OK = lowest accepted value
> OK = highest accepted value

Annex 2 z-scores of all participants - April 2013

z-scores were calculated according to the formula : $z = (x-m)/s$.

x = result of the individual laboratory.

m = mean of the results of all participating laboratories . s = standard deviation of the results of all participating laboratories .

Correct negative results in quantitative analyses obtained a z-score of zero.

False results did not generate a z-score. ■ $2 < |z| \leq 3$, ■ $|z| > 3$

Lab no	Vial	Aerobic microorganismer 30 °C			Enterobacteriaceae			Escherichia coli			Presumptive Bacillus cereus			coagulase-pos Staphylococci			Lactic acid bacteria			Clostridium perfringens			Anaerobic sulphite-reducing bacteria			Psychrotrophic microorganisms			Aerobic m.o. in fish products, 20-25 °C			H ₂ S prod. bacteria in fish products			Yeast			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				
6971	3 2 1	-2.143	1.195	0.569	0.789	0	0.396				-0.439	0																			0	0	-0.068	-2.256	0	0	6971	
7024	3 1 2	0.854	0.787	0.204	0.474	0	1.102				-0.199	0	0																	0	0	0.416	0.343	0	0	7024		
7096	3 2 1	-0.936	0.039	0.716							0	0	0.968																								7096	
7182	1 3 2	1.145	0.787	1.300	1.230	0	0.617				0	0	0.120																							7182		
7191	3 2 1	-0.353	-0.845	0.277	1.167	0	0.352				1.388	0	0																							7191		
7207	1 2 2	-0.644	0.175	0.935							-2.005	-0.309	0	0																						7207		
7232	3 2 1	-2.018	0.515	0.569							0	0	0.443																							7232		
7242	3 2 1	0.650	-0.090	0.043	0.745	0	-1.209				-0.309	0	0																						7242			
7248	2 1 3	0.105	-0.505	0.496	-0.219	0	-0.089				0	0	0.443	-1.305	0	0																		7248				
7253	3 2 1	-0.849	0	-0.045	0	0	-0.567				-0.632	0	0	0	-0.016	0	0																	7253				
7282	1 2 3	-0.062	-0.369	-0.235	0.978	0	1.190				0	0	0.200	-0.295	0	0	0	0.114	0	-0.414	0	0											7282					
7330	3 2 1	0.063	2.962	2.836	0.978	0	1.234				0	0	0.281	1.388	0	0	0	1.341	0	0.679	0	0											7330					
7334	1 3 2	0.396	0.447	-0.067							0	0	-0.276	0	0																			7334				
7438	3 2 1	-0.145	-1.117	0.642	-0.408	0	0.970				0	0	0.402	-0.632	0	0	0	-1.824	0														7438					
7449	1 2 3	0.147	0.243	0.204	-0.408	0	-0.751				0	0	-0.527	1.004	0	0	0	-0.791	0														7449					
7543	3 2 1	-0.062	-0.097	0.642							0	0	-0.567	1.485	0	0	0	-0.274	0	1.117	0	0	0	0.321	0	-1.174	-0.964	1.152	0.549			7543						
7564	1 3 2	0.604	-0.573	-0.162	-2.299	0	-4.000				0	0	-0.567	1.100	0	0	0	-2.083	0								-1.051	-2.003	0	0	0	0.597	0.943	0	0	7564		
7596	1 2 3	-1.560	0.107	1.008	0.978	0	1.014				0	0	0.402	0	0			-0.824	0	0															7596			
7627	2 3 1	0.063	0.379	-0.235							0	0	0.847	-0.439	0	0	0	0.372	0	0.388	0	0	0	1.106	0	0	-0.109	0	0.391	-0.124	0.522			7627				
7631	1 3 2	0.271	-0.369	-0.016							0	0	1.312	0.311	0.058	0	0	0.1372	0	-0.985	0	0	0	-0.497	0	0	-1.215	0	0	0	0.113	0.410	0	0	7631			
7688	2 3 1										0	0	-4.000	-0.343	0	0	0	-0.339	0	0.752	0	0	0	0.088	0										7688			
7728	3 2 1	1.312	0.311	0.058							0	0	0.039	-0.151	0	0	0	0.400	0	-0.043	0	0	0	-1.121	0										7728			
7750	3 1 2	-0.561	-0.505	-0.747							0	0	-4.000	-0.343	0	0	0	-0.339	0	0.752	0	0	0	0.088	0										7750			
7793	1 3 2	-0.228	-0.845	0.131	0.978	0	0.617				0	0	0.039	-0.151	0	0	0	0.400	0	-0.043	0	0	0	-1.121	0									7793				
7825	3 1 2	0.201	0.916	0.452	-0.062		-2.920				0	0	1.061																					7825				
7828	1 3 2	0.854	0.515	0.496	0.978	0	-0.618				0	0	-0.810	1.581	0	0	0	0.501	0														7828					
7876	1 3 2	0.105	0.787	1.008	-0.282	0	-0.309				0	0	0.282	0	0	0	0	0.307	0	0	0	0	0.260	0	0	0.553	0						7876					
7906	1 2 3	-1.560	0.787	0.642	0.789	0	0.529				0	0	0.645	0	0	0	0	-0.791	0	-2.820	0	0	0	-1.726	0								7906					
7930	1 2 3	0.355	-0.981	0.277	0.348	0	1.014				0	0	1.291	0	0	0	0	0.307	0	0	0	0	0.304	0	0	0.228	0						7930					
7940	2 1 3	0.646	0.923	0.862							0	0	-0.483	0	0	0	0	-0.920	0	0	0	0	-0.372	0	0	0.514	3.319	1.016				7940						
7946	3 1 2	0.629	1.120	0.694							0	0	-0.583	0	0	0	0	4.000	0	-1.689	0	0	0	0	0	0	0	-0.195	1.303	0	0		7946					
7962	2 1 3	0.063	-0.165	0.350	0.285	0	0.264				0	0	0.604	0.811	0	0	0	-0.080	0								0	0	-2.730	-0.390	0	0	7962					
8066	1 2 3	0.537	0	-0.265	0	0	-0.406				0	0	0.366	0	0	0	0	3.666	0	-1.872	0	0	0	-0.796	0									8066				
8068	1 2 3	0.355	-0.369	0.423	1.545	0	0.970				0	0	1.089	0.138	0	0	0	3.472	0	0.388	0	0	0	0.661	0	0	-1.726	0					8068					
8105	3 1 2	-1.643	-0.165	-0.089							0	0	-0.483	0	0	0	0	-0.920	0	0	0	0	-0.372	0	0	0	0.295	0.610	0	0		8105						
8255	1 2 3	-0.936	-3.564	0.350	0.726	0	-2.162				0	0	-3.112	-1.064	0	0	0	-2.820	0	0	0	0	-1.077	0	0	0	0	0	0	0	0	8255						
8260	3 1 2	-0.436	-1.728	-1.916	-0.030	0	-0.706				0	0	-0.123	-0.343	0	0	0	-2.535	0	0	0	0	-0.114	0	0	0	0	0	0	0		8260						
8313	2 1 3	-0.003	0.216	-0.951	-0.509	0	-1.355				0	0	-0.939	0.778	0	0	0	-1.114	0	0	0	0	-0.474	0	0	0	0	0	0	0	0	8313						
8333	1 3 2	0.438	-0.029	0.423	0.600	0	0.043				0	0	-0.244	0.234	0	0	0	0.169	0	0	0	0	-0.305	0	0	0	0	0	0	0	0	8333						
8352	3 1 2	1.187	1.331	0.204	-1.038	0	-0.883				0	0	-0.931	-1.305	0	0	0	-0.274	0	-0.341	0	0	0	0	0	0	0	0	0	0	0	8352						
8380	3 1 2	0.105	-0.029	-0.089	-2.236	0	-0.927				0	0	0.079	-0.487	0	0	0	-0.1372	0	-0.633	0	0	0	0.304	0	0	0.228	0				8380						
8397	1 2 3										0	0	-0.402	1.677	0	0	0	0.759	0	-0.414	0	0	0	1.329	0	0	0.972	0						8397				
8428	3 2 1	0.188	0.107	0.423	-0.534	0	1.102				0	0	-0.274	0	1.335	0	0	0	-0.542	0	0	0	0.274	0									8428					

Lab no	Vial	Aerobic microorganismer 30 °C			Enterobacteriaceae			Escherichia coli			Presumptive Bacillus cereus			coagulase-pos Staphylococci			Lactic acid bacteria			Clostridium perfringens			Anaerobic sulphite-reducing bacteria			Psychrotrophic microorganisms			Aerobic m.o. in fish products, 20-25 °C			H ₂ S prod. bacteria in fish products			Yeast			Lab no						
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C							
9217	3 1 2	-0.395	-1.049	0.277	0.285	0	0.705				-0.968	0																			0	0	-4.000	-0.856	0	9217								
9245	1 2 3	0.147	-0.437	-0.089	-1.164	0	-0.839				1.773	0	0																	0	0	1.202	0.010	0	9245									
9420	1 2 3	0.105	0.447	0.350	0.159	0	0.308	0	0	0.645																										9420								
9429	2 1 3	0.937	0.787	0.862	-0.030	0	-0.133	0	0	-0.365	-1.064	0	0	0	0.695	0		0	1.195	0	0	0	0.321	0										0	0	0.597	1.077	0	9429					
9436	2 1 3	0.812	0.719	4.000	0.159	0	-1.368	0	0	0.160	0.090	0	0	0	-0.791	0	-2.456	0	-0.720	0	0	0	0.321	0	1.370	-1.019	0.101										9436							
9441	3 1 2	0.355	0.447	0.350	-0.597	0	-0.398	0	0	0.725	-0.103	0	0	0	0.108	0	0.606	0	0	0.839	0	0	0.460	0	-0.489	-0.180	-0.865	0.384	0.073	-0.242	-0.263	0.166	0	0	0	0.839	-0.057	0	9441					
9451	2 3 1	0.147	-0.369	0.131	-0.534	0	0.440	0	0	0.160	1.004	0	0	0	-0.533	0		0	0.260	0	0	0.786	0	0.570	-1.870	0.866											9451							
9453	1 3 2	0.230	-0.097	-0.016	0.222	0	-0.927				-1.738	0	0	0	0	0.114	0	0.388																0	0	0.658	-0.323	0	9453					
9465	3 2 1																																					9465						
9512	2 1 3	0.479	0.175	-1.039	-0.408	-0.662				0.811	0																								0	-2.125	-0.390	0	0	9512				
9555	1 2 3	-0.020	0.107	0.131	-1.353	0	-0.442	0	0	-0.527	-0.054	0	0	0	-1.049	0		0	-0.408	0												0	0	0.113	0.077	0	0	9555						
9559	3 1 2	-0.686	-0.165	0.569	0.285	-0.265	0	0	-0.688	-0.151	0	0	0	0.372	0	-4.000	0																0	0	1.081	-1.389	0	0	9559					
9569	3 2 1	-0.103	0.107	-0.162	-0.282	0	0.793	0	0	0.321	-0.824	0	0	0	-0.533	0	-0.414	0	0	-0.008	0	0	0.321	0				0.616	1.557	-0.163								9569						
9589	3 1 2	-0.228	-0.369	-0.527	0.159	-1.059	0	0	-1.092	-1.064	0	0	0	-0.920	0	0.388	0	0	0	-1.923	0										0	0	-1.520	-1.589	0	0	9589							
9662	3 1 2	4.000	0.379	3.055	1.167	0	0.308	0	0	-0.204	-0.439	0	0	-4.000	0	0.752	0	0	0	-0.497	0	0	0	-0.982	0									0	0	-4.000	0.277	0	0	9662				
9747	3 2 1	-0.311	-0.369	-0.747	4.000	0	-1.456				-1.882	0	0																			0	0	0.779	-1.123	0	0	9747						
9783	2 1 3	-1.722	0.433	-0.059																																		9783						
9886	3 1 2	0.604	0.719	1.081	-1.164	0	0.793	0	0	0.685	-0.632	0	0	0	0.759	0	0.388	0	0	0.393	0	0	0.879	0	0.294	-0.893	-1.177									0	0	-0.733	1.410	0	0	9886		
9890	1 2 3	0.979	-0.709	0.277	2.112	0.881	0	0	0.806	-0.487	0	0	0	3.925	0	0.388	0																0	0	0.779	-0.323	0	0	9890					
9903	3 1 2	-0.270	0.311	-0.308	0.474	0	0.220	0	0	0.564	0.186	0	0	0	0.824	0		0	0.260	0				0.881	-0.865									0	0	0.355	-0.057	0	0	9903				
9923	3 1 2	-0.603	-1.728	-1.697	-1.479	0	-1.015	0	0	-1.052				-4.000	0	0	-0.706																	0	0	-0.915	0.410	0	0	9923				
9950	3 1 2	0.147	-3.088	-0.016																																		0	0	0.234	0.677	0	0	9950

1. Fisk, skaldjur och fiskprodukter – analys av näringsämnen av V Öhrvik, A von Malmborg, I Mattisson, S Wretling och C Åstrand.
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19. Från nutritionsforskning till kostråd – så arbetar Livsmedelsverket av I Mattisson, H Eneroth och W Becker.
20. Proficiency Testing – Food Microbiology, October 2012 by L Nachin ,C Normark and I Boriak
21. Dioxin- och PCB-halter i fisk och andra livsmedel 2000-2011 av T Cantillana och M Aune.
22. Not publiced.
23. Kontroll av kontaminanter i livsmedel 2011 – Resultat från kontrollprogrammen för dioxiner och dioxinlikna PCB, PAH, nitrat, mykotoxiner och tungmetaller av A Wannberg, F Broman och H Omberg.
24. Proficiency Testing – Drinking Water Microbiology, 2012:2, September by T Šlapokas and K Mykkänen.

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Contaminants and minerals in foods for infants and young children – risk and benefit assessment, Part 2 by G Concha, H Enerothe, H Hallström and S Sand.
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