

Food Microbiology

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Proficiency Testing
Microbiology – Food
April 2016



1457
ISO/IEC 17043

Quantitative analyses

- Aerobic microorganisms, 30 °C
- Psychrotrophic microorganisms
- 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
BA-P	Blood Agar including Polymyxin
BcsA	<i>Bacillus cereus</i> -selective Agar
BcsA-P	<i>Bacillus cereus</i> -selective Agar including Polymyxin
BP	Baird-Parker agar
BP + RPFA	Baird-Parker-agar with Rabbit Plasma Fibrinogen
DG18	Dichloran Glycerolagar
DRBC	Dichloran Rose Bengal Chloramphenicol agar
ISA	Iron Sulphite Agar
LTLSB	Lactose Tryptone Lauryl Sulphate Broth
mCP	Membrane- <i>Clostridium perfringens</i> 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	Manitol egg Yolk Polymyxin agar
OGYE	Oxytetracyclin Glucose Yeast Extract agar
PAB	Perfringens Agar Base
PCA	Plate Count Agar
RPFA	Rabbit Plasma Fibrinogen Agar
SC	Sulphite Cycloserin agar
SFP	Shahidi Ferguson Perfringens agar base
TBX	Trypton Bile X-glucuronide 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

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 provide method information for all analyses for which they report results. Method information is sometimes difficult to interpret, e.g. when a medium that is not mentioned in the standard method is stated. Results from laboratories with ambiguous details are either excluded or placed in the group "Other/Unknown" in the tables, together with results from methods used only by individual laboratories

Uncertainty of measurement for the assigned values

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




Tables and figures legend

Tables

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

Figures

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

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

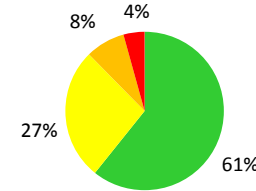
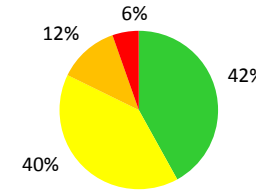
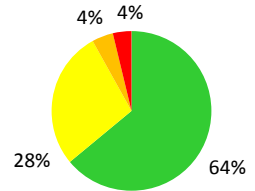
Results of the PT round April 2016

General outcome

Samples were sent to 201 laboratories, 45 in Sweden, 134 in other European countries, and 22 outside Europe. Of the 186 laboratories that reported evaluated results, 150 (81 %) provided at least one result that received an annotation. In the previous round (April 2015) with similar analyses, the proportion was 72 %.

Individual results are available on the website after logging in: www2.slv.se/absint.

Table 1. Mixtures content and % of deviating results (F: false result, O: outliers).

	Mixture A			Mixture B			Mixture C		
% of participants with									
Organisms	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Lactobacillus plantarum</i> <i>Clostridium perfringens</i> <i>Candida glabrata</i> <i>Cladosporium cladosporioides</i>			<i>Hafnia alvei</i> <i>Bacillus cereus</i> <i>Carnobacterium piscicola</i> <i>Clostridium bifermentans</i> <i>Penicillium verrucosum</i>			<i>Staphylococcus saprophyticus</i> <i>Escherichia coli</i> <i>Bacillus thuringiensis</i> <i>Shewanella putrefaciens</i>		
Analysis	Target organism	F %	O %	Target organism	F %	O %	Target organism	F %	O %
Aerobic micro-organisms, 30 °C	<i>P. aeruginosa</i> <i>L. plantarum</i>	1	6	<i>H. alvei</i> <i>C. piscicola</i>	0	5	<i>S. saprophyticus</i>	0	4
Psychrotrophic microorganisms	<i>C. cladosporioides</i>	69	0	<i>H. alvei</i> <i>C. piscicola</i>	0	7	-	0	0
Enterobacteriaceae	(<i>P. aeruginosa</i>)	7	-	<i>H. alvei</i>	1	7	<i>E. coli</i>	0	9
<i>E. coli</i>	-	1	-	-	4	-	<i>E. coli</i>	16	6
Presump. <i>B. cereus</i>	-	3	-	<i>B. cereus</i>	32	1	<i>B. thuringiensis</i>	2	7
Coagulase-positive Staphylococci	<i>S. aureus</i>	1	7	-	1	-	(<i>S. saprophyticus</i>)	1	-
Lactic acid bacteria	<i>L. plantarum</i>	2	2	<i>C. piscicola</i>	66	0	(<i>S. saprophyticus</i>)	17	-
<i>C. perfringens</i>	<i>C. perfringens</i>	3	9	-	19	0	-	0	-
Anaerobic sulphite-reducing bacteria	<i>C. perfringens</i>	1	3	<i>C. bifermentans</i>	9	0	-	0	-
Aerobic micro-organisms in fish products, 20-25 °C	<i>P. aeruginosa</i> <i>L. plantarum</i>	3	3	<i>H. alvei</i> <i>C. piscicola</i>	0	6	<i>S. saprophyticus</i>	0	6
H ₂ S-producing bacteria in fish prod.	-	3	-	<i>H. alvei</i>	3	3	<i>S. putrefaciens</i>	48	0
Yeasts	<i>C. glabrata</i>	0	16	-	6	-	-	0	-
Moulds	<i>C. cladosporioides</i>	11	4	<i>P. verrucosum</i>	5	6	-	1	-

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

Aerobic microorganisms, 30 °C

Mixture A

Strains of *Pseudomonas aeruginosa* and *Lactobacillus plantarum* were present in the highest concentrations in mixture A, and thus the majority of the colonies were from these species.

Mixture B

Strains of *Hafnia alvei* and *Carnobacterium piscicola* were present in the highest concentrations in mixture B, and thus the majority of the colonies were from these species.

Mixture C

A strain of *Staphylococcus saprophyticus* was present in the highest concentration, and thus the majority of the colonies were from this species.

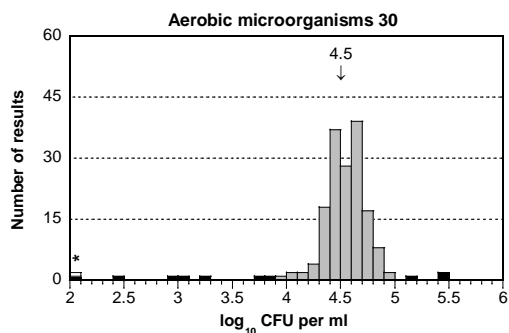
General remarks

The results from all three mixtures were well distributed, though the results for mixture C had a somewhat wider distribution compared to mixtures A and B. Only a few high and low outliers were reported.

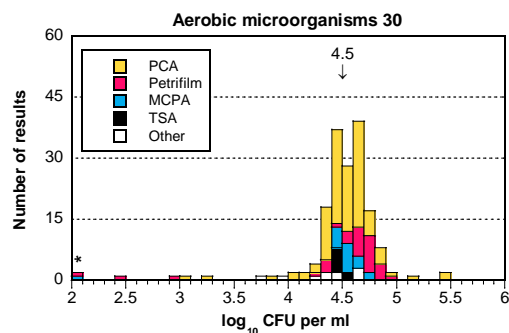
Results of aerobic microorganisms analysis

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	171	158	4.54	0.17	1	7	3	162	4.71	0.14	0	7	2	164	4.55	0.30	0	6	0
PCA	103	95	4.52	0.17	0	3	3	100	4.69	0.14	0	3	0	102	4.55	0.30	0	0	0
Petrifilm™	32	29	4.65	0.17	0	3	0	28	4.76	0.11	0	3	1	27	4.60	0.26	0	5	0
MCPA	18	17	4.56	0.10	1	0	0	17	4.69	0.10	0	1	0	17	4.56	0.21	0	1	0
TSA	8	8	4.48	0.05	0	0	0	8	4.67	0.12	0	0	0	8	4.39	0.30	0	0	0
Other	10	9	4.42	0.22	0	1	0	9	4.80	0.20	0	0	1	10	4.62	0.43	0	0	0

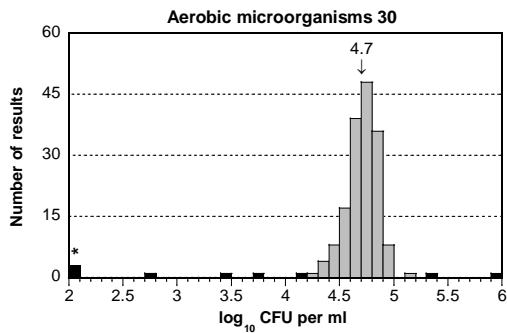
A



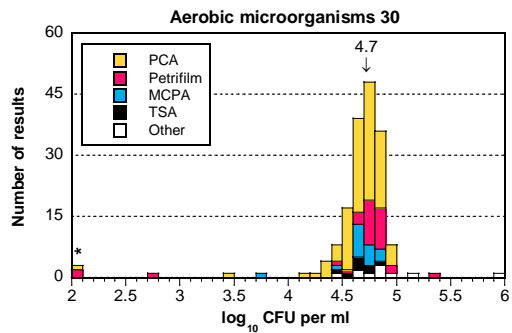
A



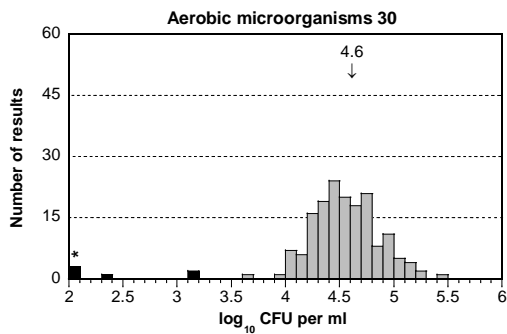
B



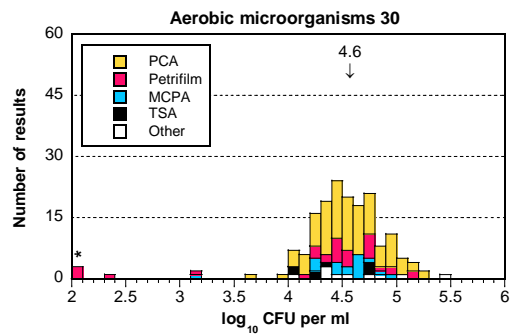
B



C



C



Psychrotrophic microorganisms

Mixture A

At the National Food Agency *Cladosporium cladosporioides* formed colonies on PCA after 10 days incubation at 6.5 °C (NMKL 86:2013). The colonies were very small, and a magnifying glass was used for enumeration, which may explain why 9 of the 13 laboratories that performed the analysis reported false negative results. These false negatives could not be associated with the use any specific method, media or incubation temperature.

Mixture B

As in the analysis of aerobic microorganisms at 30 °C, strains of *Hafnia alvei* and *Carnobacterium piscicola* were target organisms for the analysis, and thus the majority of the colonies were from these two species. The only divergent result was a low outlier.

Mixture C

Mixture C contained no target organism for this analysis. At the National Food Agency, neither of the strains present in the mixture formed colonies on PCA after 10 days incubation at 6.5 °C. However at higher temperatures, which is recommended by some methods, microorganisms present in the mixture can grow. Therefore, both positive and negative results are considered correct for this analysis. As a consequence, the results have not been evaluated, and no z-scores have been calculated. The results are also excluded from the tables located below the box plots.

General remarks

All laboratories except two used PCA or MCPA for the cultivation. Incubation times and temperatures varied, as a result of differences among the methods that were followed. Incubation time and temperature therefore differed between 6.5 °C / 10 days (NMKL 86:2006), 17 °C / 20 h + 7 °C / 3 days (NMKL 74:2000) and 21 °C / 24 h (ISO 8552:2004). Furthermore NMKL 86:2013, which has replaced NMKL 86:2006 and NMKL 74:2000 prescribes incubation either at 6.5 °C / 10 days, or at 17 °C / 20 h, followed by 7 °C / 3 days. These differences in incubation time and temperature may explain the large variation in results observed between laboratories, but also raises the question of the definition of psychrotrophic microorganisms. With that in mind, it should also be noted that for mixture C, there was a slight trend for higher incubation temperatures to coincide with higher numbers of reported microorganisms.

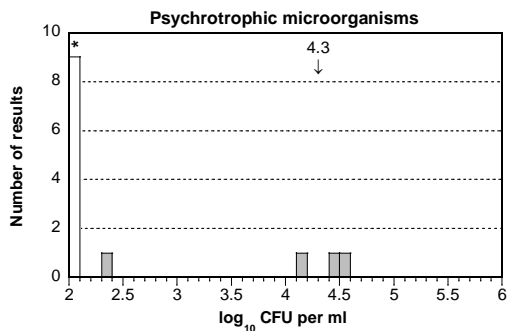
The variations in incubation time and temperature, combined with a low number of participants, makes it difficult to statistically evaluate the results. As a consequence, median values are provided instead of mean values in the table below.

Results of psychrotrophic microorganisms analysis

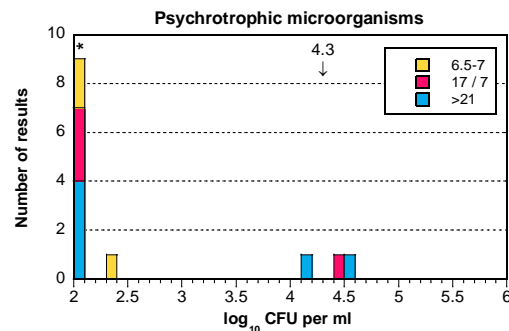
Temperature	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	14	4	4.31*	-	9	-	-	13	4.60*	-	0	1	0	13	3.99*	-	0	-	-
6.5–7 °C	3	1	2.34*	-	2	-	-	3	4.67*	-	0	0	0	2	0*	-	0	-	-
17 °C / 7 °C	4	1	4.48*	-	3	-	-	4	4.55*	-	0	0	0	4	2.78*	-	0	-	-
≥21 °C	7	2	4.37*	-	4	-	-	6	4.47*	-	0	1	0	7	4.30*	-	0	-	-

* Median

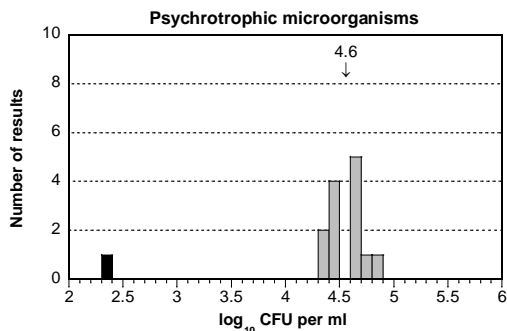
A



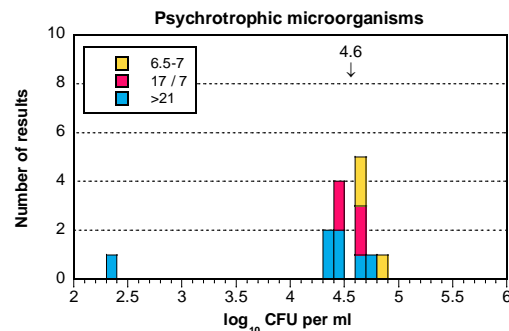
A

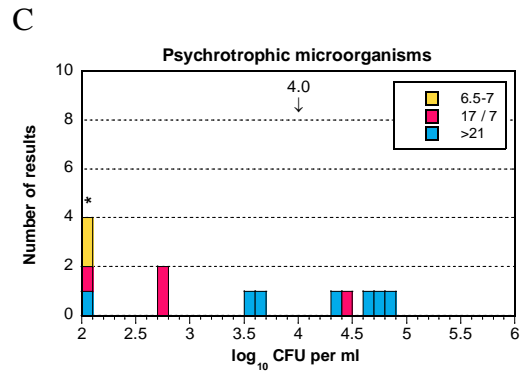
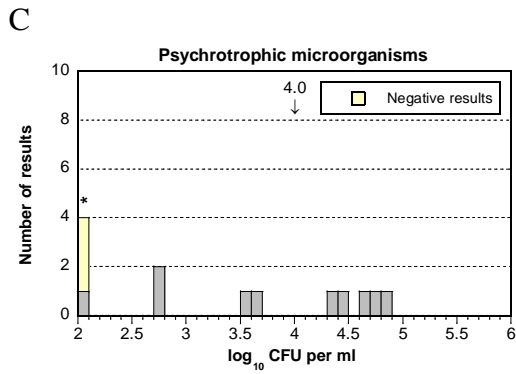


B



B





Enterobacteriaceae

Mixture A

Mixture A contained no target organism for this analysis. At the National Food Agency, *Pseudomonas aeruginosa* formed small, atypical, and beige colonies on VRBG. This could explain why 11 laboratories reported false positive results for Enterobacteriaceae, especially considering that only 2 of these performed a confirmation test.

Mixture B

A strain of *Hafnia alvei* was target organism for the analysis. The results were well distributed, with a few outliers and one false negative result.

Mixture C

A strain of *Escherichia coli* was target organism for the analysis. The results were well distributed, with a small number of outliers.

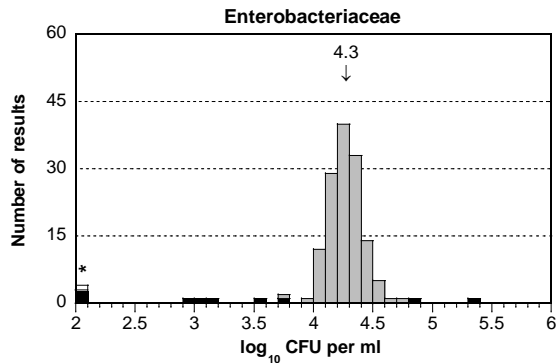
General remarks

Most laboratories used VRBG or 3M™ Petrifilm™ Enterobacteriaceae (Petrifilm™) for the analysis, and concordant results were also reported for these two media.

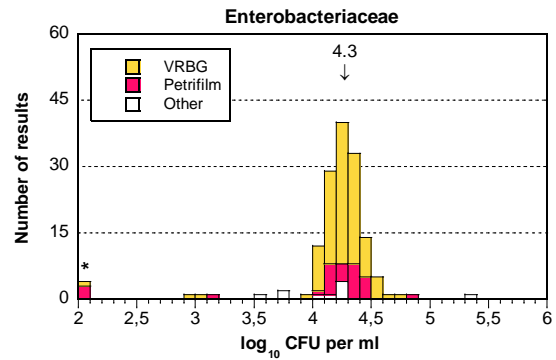
Results of Enterobacteriaceae analysis

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	148	136	-	-	11	-	-	137	4.27	0.14	1	8	2	134	3.09	0.11	0	6	7
VRBG	108	99	-	-	8	-	-	105	4.27	0.14	0	3	0	101	3.08	0.11	0	3	3
Petrifilm™	30	27	-	-	3	-	-	25	4.29	0.11	1	3	1	25	3.12	0.09	0	3	2
Other	10	10	-	-	-	-	-	7	4.16	0.17	0	2	1	8	3.07	0.07	0	0	2

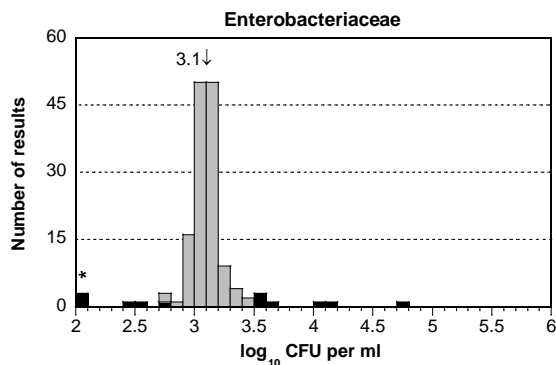
B



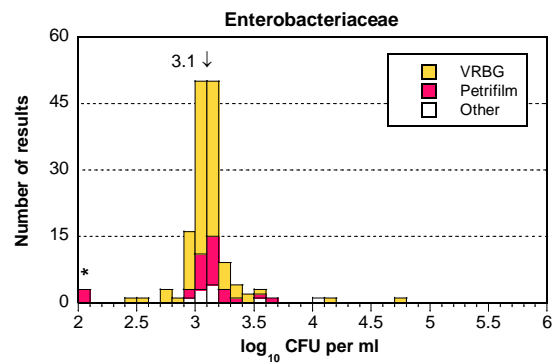
B



C



C



Escherichia coli

Mixture A

No target organism for the analysis of *E. coli* was present in mixture A. Only one false positive result was reported.

Mixture B

No target organism for the analysis of *E. coli* was present in mixture B. Despite this, 5 of 122 laboratories reported false positive results.

Mixture C

A strain of *E. coli* was target organism for the analysis. False negative results were reported by 19 laboratories. Of these, 11 used 3M™ Petrifilm™ *E. coli*/Coliform count plate (Petrifilm™ EC/CC), which may indicate that the strain of *E. coli* was difficult to identify with this method.

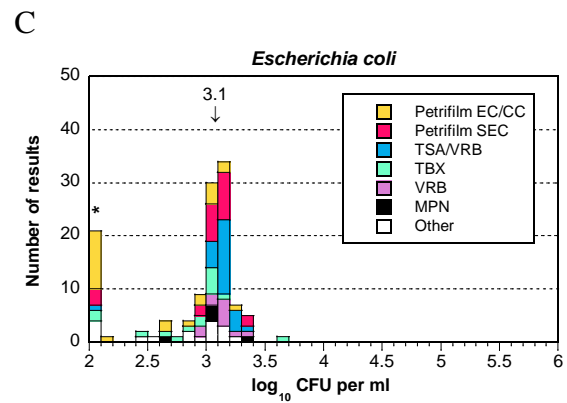
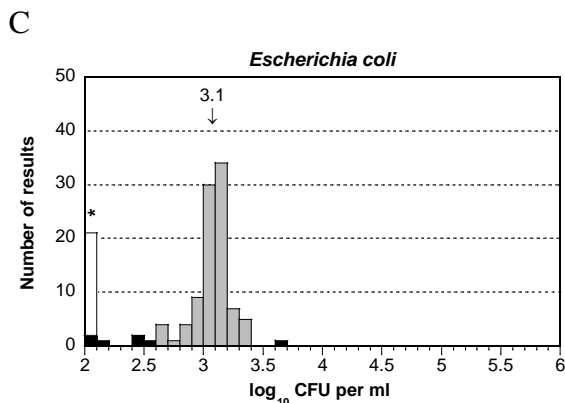
General remarks

A fairly large assortment of media were used for the analysis of *E. coli*, though concordant results were still reported independent of the media used. The majority of the false positive and false negative results are likely a consequence of not performing a confirmation test, or of problems in carrying out the confirmation. For the analysis of *E. coli*, NMKL 125:2005 recommends confirming presumptive colonies by testing production of gas and indole in LTL5B at 44 °C, whereas confirmation in 3M™ Petrifilm™ Select *E. coli* Count (Petrifilm™ SEC) and ISO 16649-2:2001 is based on

detection of β -glucuronidase activity in *E. coli* at 42 °C and 44 °C respectively. Petrifilm™ EC/CC, from which most of the false negative results originated, includes detection of both β -glucuronidase activity and production of gas, but is in contrast performed at 37 °C. At the National Food Agency, the present strain of *E. coli* is noted for displaying a weak β -glucuronidase activity, and it may have been difficult to distinguish from other coliform bacteria when using Petrifilm™ EC/CC.

Results of *E.coli* analysis

Media	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	122	120	-	-	1	-	-	117	-	-	5	-	-	94	3.08	0.15	19	6	1
Petrifilm™ EC/CC	24	23	-	-	0	-	-	22	-	-	2	-	-	12	2.98	0.20	11	1	0
Petrifilm™ SEC	23	22	-	-	1	-	-	23	-	-	0	-	-	20	3.11	0.10	2	1	0
TSA/VRB	25	25	-	-	0	-	-	25	-	-	0	-	-	24	3.15	0.09	1	0	0
TBX	15	15	-	-	0	-	-	15	-	-	0	-	-	11	2.96	0.15	1	2	1
VRB	11	11	-	-	0	-	-	10	-	-	1	-	-	11	3.12	0.10	0	0	0
MPN	7	7	-	-	0	-	-	7	-	-	0	-	-	5	3.03	0.25	0	0	0
Other	24	17	-	-	0	-	-	15	-	-	2	-	-	11	3.05	0.13	4	2	0



Presumptive *Bacillus cereus*

Mixture A

No target organism for this analysis was present in mixture A. Despite this, 4 laboratories reported false positive results. Three of these used BA and followed NMKL 67:2010, which stipulates confirmation of suspected colonies from BA on BcsA or Cereus-Ident agar (a chromogenic media). However none of these laboratories reported performing such a confirmation test.

Mixture B

A strain of *Bacillus cereus* was target organism for this analysis. This particular strain forms atypical shiny colonies with a small zone of haemolysis on BA. On BcsA it forms light blue colonies with a faint zone of precipitation. The atypical appearance likely contributed to the fact that 41 of 127 laboratories reported false negative results. These could also not be connected to the use any specific method or media.

Mixture C

A strain of *Bacillus thuringiensis*, which belongs to the group of presumptive *B. cereus*, was target organism for this analysis. The results were well distributed, with a few outliers and false negative results.

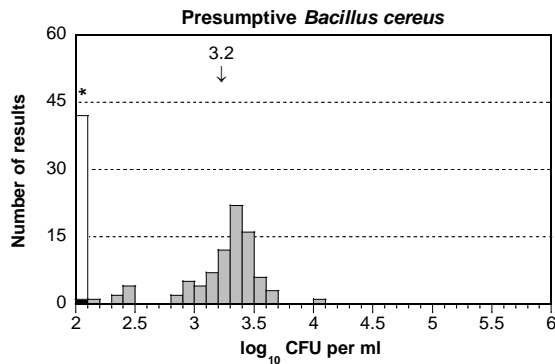
General remarks

Most laboratories reported following either NMKL 67:2010 or ISO 7923:2004. No difference in the results between these two methods could be found, neither for mixture B nor for mixture C.

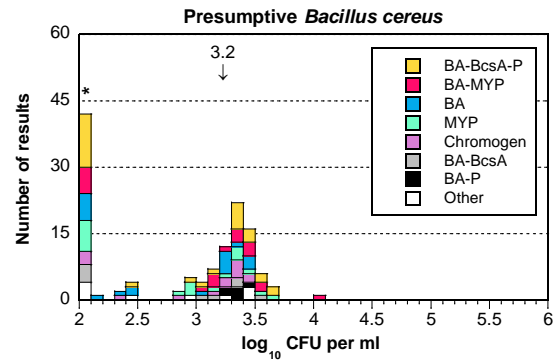
Results of presumptive *B. cereus* analysis

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	127	123	-	-	4	-	-	85	3.23	0.32	41	1	0	115	3.14	0.20	3	6	3
BA-BcsA-P	28	28	-	-	0	-	-	17	3.31	0.28	12	0	0	29	3.16	0.17	0	0	0
BA-MYP	21	21	-	-	0	-	-	14	3.37	0.24	6	0	0	18	3.13	0.21	0	1	2
BA	20	17	-	-	3	-	-	14	3.04	0.46	6	0	0	15	3.06	0.19	2	2	0
MYP	19	19	-	-	0	-	-	12	3.22	0.27	7	0	0	18	3.11	0.19	0	0	1
Chromogen	14	14	-	-	0	-	-	11	3.19	0.33	2	1	0	12	3.21	0.18	0	2	0
BA-BcsA	9	9	-	-	0	-	-	5	3.28	0.21	4	0	0	9	3.19	0.27	0	0	0
BA-P	6	5	-	-	1	-	-	6	3.30	0.08	0	0	0	6	3.15	0.23	0	0	0
Other	10	10	-	-	0	-	-	6	3.16	0.40	4	0	0	8	3.11	0.27	1	1	0

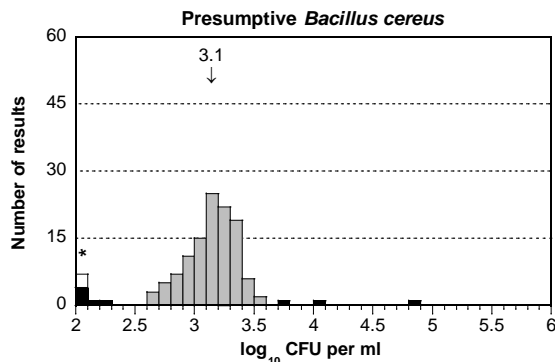
B



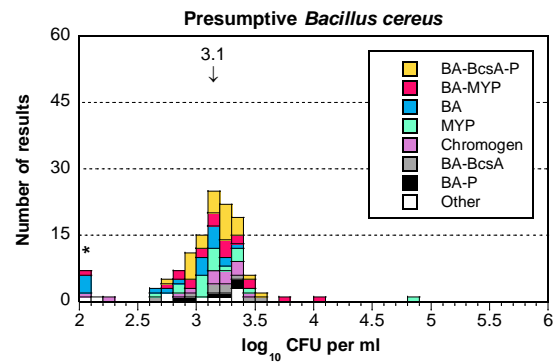
B



C



C



Coagulase-positive *Staphylococci*

Mixture A

A strain of *Staphylococcus aureus* was target organism for this analysis. The results had a good distribution, and only a few laboratories reported outliers.

Mixture B

Mixture B contained no target organism for this analysis. Only one laboratory reported a false positive result.

Mixture C

Mixture C contained no coagulase-positive strain of *Staphylococci*. Only one laboratory reported a false positive result.

General remarks

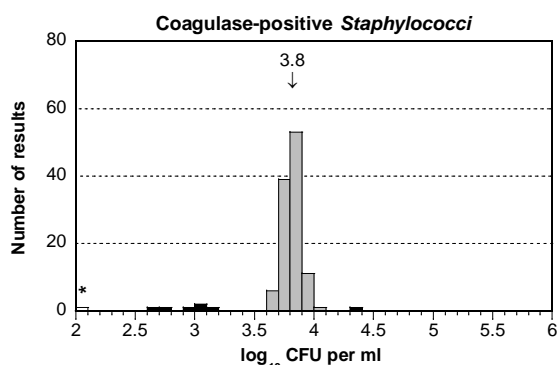
The analyses were unproblematic for the laboratories, and no divergence could be detected among the different media and methods that were used. In earlier PT rounds, high outliers and false positive results have been associated with the use of BP agar. This media does not test for coagulase activity, and additional confirmation tests are therefore required. In the present PT round, only a few outliers and false positive results were reported, likely due to the fact that 67 of the 70 laboratories that used BP agar performed additional confirmation tests.

Results of coagulase-positive *Staphylococci* analysis

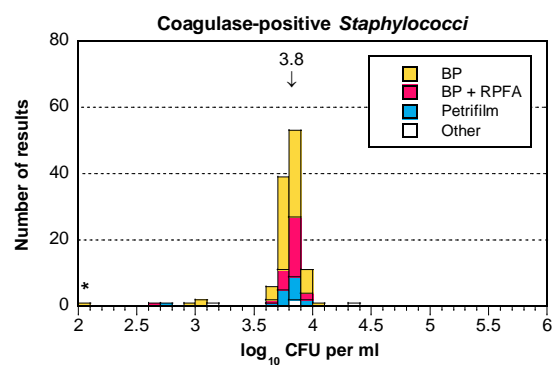
Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	120	110	3.81	0.07	1	6	2	119	-	-	1	-	-	118	-	-	1	-	-
BP	70	66	3.81	0.07	1	3	0	70	-	-	0	-	-	70	-	-	0	-	-
BP + RPFA	29	27	3.82	0.06	0	1	0	29	-	-	0	-	-	29	-	-	0	-	-
Petrifilm™	17	15	3.81	0.07	0	1	1	16	-	-	1	-	-	15	-	-	1	-	-
Other	4	2	3.87*	-	0	1	1	4	-	-	0	-	-	4	-	-	0	-	-

* Median

A



A



Lactic acid bacteria

Mixture A

A strain of *Lactobacillus plantarum* was target organism for the analysis. The counting of colonies was unproblematic, and the results were well distributed. Only two deviating results were reported. No divergence due to the use of different media or methods could be found.

Mixture B

A strain of *Carnobacterium piscicola* was target organism for the analysis. This strain is more sensitive to low pH compared to other lactic acid bacteria, which may explain why 38 of 58 laboratories reported false negative results. Due to the difficulties associated with identifying the strain, all positive results are considered acceptable. As a consequence, the widely distributed positive results also have high standard deviations. The mean value for MRS is also fairly low, due to the inclusion of two very low results.

Mixture C

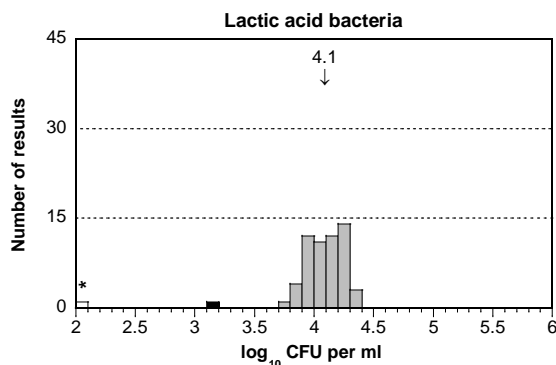
Mixture C contained no target organism for this analysis. Nevertheless, at the National Food Agency, *Staphylococcus saprophyticus* formed small colonies on MRS-aB after 5 days of anaerobic incubation at 25 °C. In previous rounds, the same strain has been shown to grow also on MRS. This could explain the deviating results; 9 of the 10 laboratories that reported false positives used either MRS or MRS-aB. The majority of the reported concentrations from these laboratories also corresponded to that of *S. saprophyticus* in the mixture.

Results of lactic acid bacteria analysis

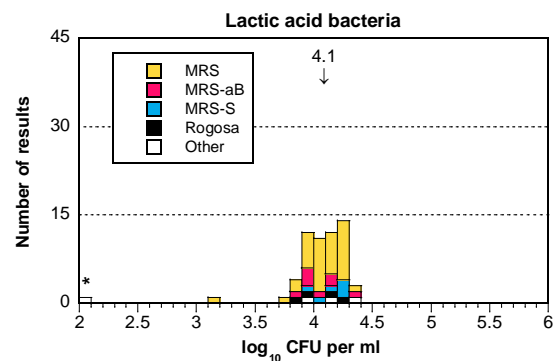
Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	59	57	4.08	0.14	1	1	0	20	4.25	0.56	38	0	0	49	-	-	10	-	-
MRS	37	36	4.06	0.21	0	1	0	13	4.12	0.63	23	0	0	29	-	-	7	-	-
MRS-aB	9	8	4.04	0.14	0	0	0	4	4.44*	-	5	0	0	7	-	-	2	-	-
MRS-S	6	6	4.11	0.12	0	0	0	2	4.58*	-	4	0	0	6	-	-	0	-	-
Rogosa	4	4	4.01*	-	0	0	0	0	-	-	4	0	0	4	-	-	0	-	-
Other	3	3	4.10*	-	1	0	0	1	4.93*	-	2	0	0	3	-	-	1	-	-

* Median

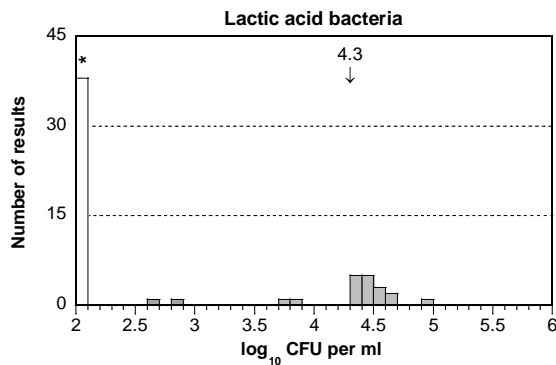
A



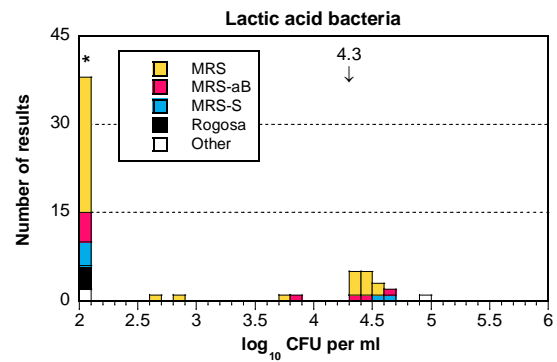
A



B



B



***C. perfringens* and anaerobic sulphite-reducing bacteria**

Mixture A

A strain of *C. perfringens* was target organism for both *C. perfringens* and for anaerobic sulphite-reducing bacteria. The results were well distributed for both analyses, but a number of low outliers were reported in the case of *C. perfringens*. NMKL 95:2009 recommends the use of TSC and/or mCP as media for *C. perfringens*, and a majority of the laboratories (79 %) reported the use of TSC. Of the 6 laboratories that used mCP, 3 reported low outliers, and 3 reported results that were noticeably lower compared to other media. This suggest that the strain was difficult to identify with mCP. It could therefore be mentioned that mCP is often used in membrane filtration analyses of water, where it has been found to result in lower recovery of *C. perfringens* compared to TSC (2, 3, 4). Comparative studies on food analyses have also advocated TSC as the preferred media for detecting *C. perfringens* (5, 6).

Mixture B

Mixture B contained a strain of *C. bifermentans*, which was target organism only for the analysis of anaerobic sulphite-reducing bacteria. It can be distinguished from *C. perfringens* in confirmation tests – unlike *C. perfringens*, *C. bifermentans* is motile. Not performing a confirmation, or experiencing problem with the confirmation, could explain why 13 of 68 laboratories reported false positive results for *C. perfringens*. The distribution of results for anaerobic sulphite-reducing bacteria was fairly wide, and 6 of 63 laboratories reported false negative results. These could not be attributed to the use of any specific media or method.

Mixture C

Mixture C contained no target organism for these analyses. No false positive results were reported for either of the analyses.

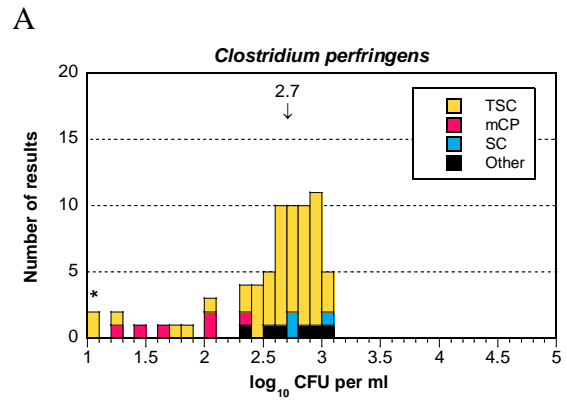
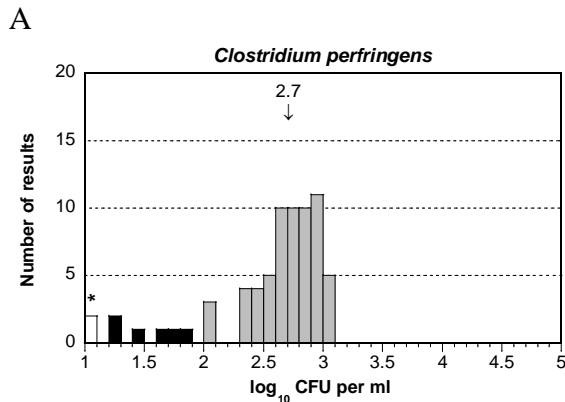
General remarks

Of the 70 laboratories that analysed *C. perfringens*, a majority followed either NMKL95:2009 or EN ISO 7937:2004. No difference in the results between these methods could be observed. For the analysis of anaerobic sulphite-reducing bacteria, the main methods used were instead NMKL 56:2008 and ISO 15213:2003; these too produced concordant results.

Results of *C. perfringens* analysis

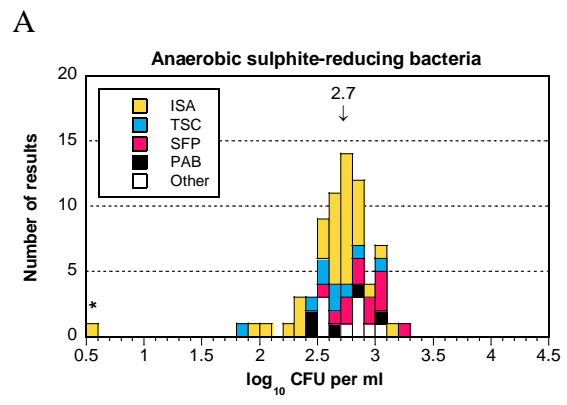
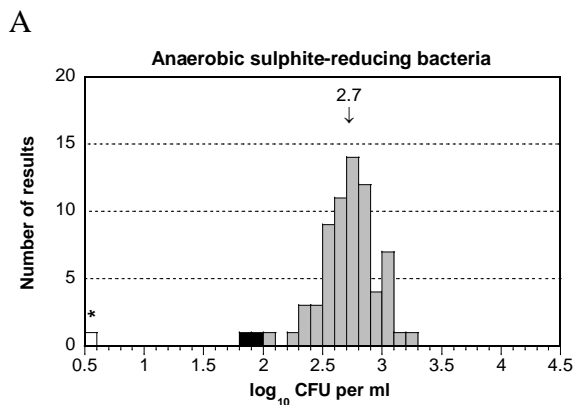
Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	70	62	2.71	0.24	2	6	0	55	-	-	13	-	-	70	-	-	0	-	-
TSC	55	50	2.73	0.20	2	3	0	44	-	-	11	-	-	54	-	-	0	-	-
mCP	6	3	2.08*	-	0	3	0	5	-	-	0	-	-	7	-	-	0	-	-
SC	3	3	2.76*	-	0	0	0	2	-	-	1	-	-	3	-	-	0	-	-
Other	6	6	2.71	0.25	0	0	0	4	-	-	1	-	-	6	-	-	0	-	-

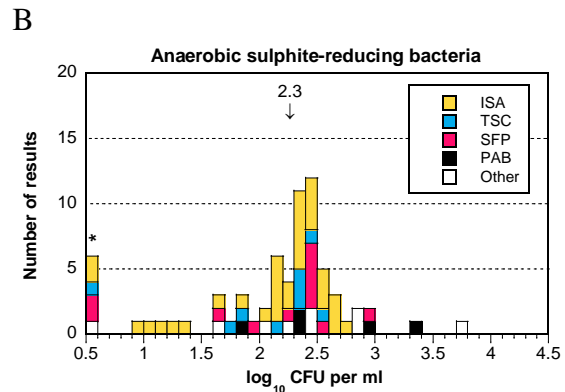
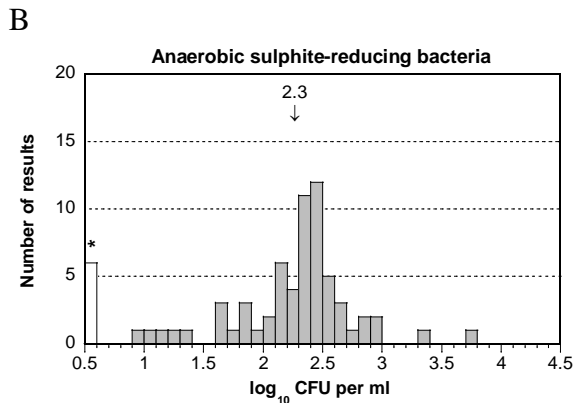
* Median



Results of anaerobic sulphite-reducing bacteria analysis

Media	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	70	67	2.72	0.22	1	2	0	63	2.26	0.50	6	0	0	69	-	-	0	-	-
ISA	35	33	2.66	0.22	1	1	0	32	2.13	0.50	2	0	0	34	-	-	0	-	-
SFP	12	12	2.88	0.19	0	0	0	10	2.34	0.35	2	0	0	12	-	-	0	-	-
TSC	9	8	2.70	0.19	0	1	0	8	2.24	0.28	1	0	0	9	-	-	0	-	-
PAB	5	5	2.70	0.26	0	0	0	5	2.54	0.58	0	0	0	5	-	-	0	-	-
Other	9	9	2.76	0.18	0	0	0	8	2.54	0.65	1	0	0	9	-	-	0	-	-





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

Mixture A

As in the analysis of aerobic microorganisms at 30 °C, strains of *Pseudomonas aeruginosa* and *Lactobacillus plantarum* were target organisms for the analysis of aerobic microorganisms in fish and fish products. Only two deviating results were reported, and the results showed a good distribution. No target organism for H₂S-producing bacteria was present in the mixture, and only one laboratory reported a false positive result for that analysis.

Mixture B

Strains of *Hafnia alvei* and *Carnobacterium piscicola* were target organisms for the analysis of aerobic microorganisms, which was unproblematic except for two outliers. For H₂S-producing bacteria, a strain of *Hafnia alvei* was target organism. Again, the results were well distributed, and only two laboratories reported deviating results.

Mixture C

As in the analysis of aerobic microorganisms at 30 °C, a strain of *Staphylococcus saprophyticus* was target organism for the analysis of aerobic microorganisms in fish and fish products. The results were well distributed, with only two outliers. A strain of *Shewanella putrefaciens* was target organism for H₂S-producing bacteria, and was present in a low concentration in the mixture. At the National Food Agency, small colonies were observed in the undiluted (0) sample. These could easily go undetected, which likely explains why half of the laboratories reported false negative results for this analysis.

General remarks

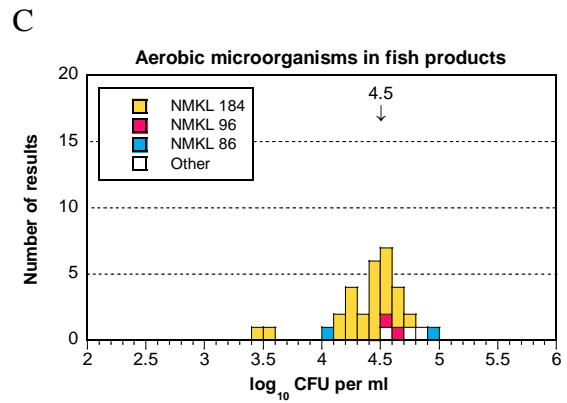
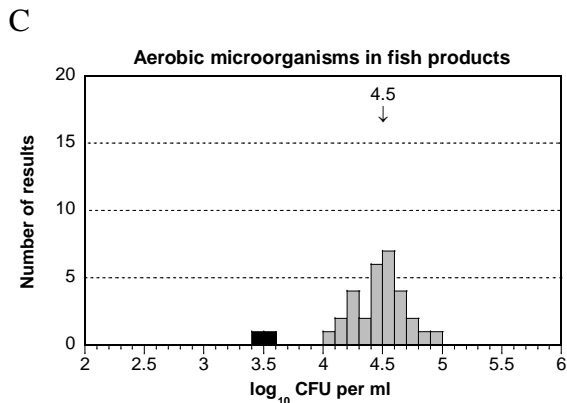
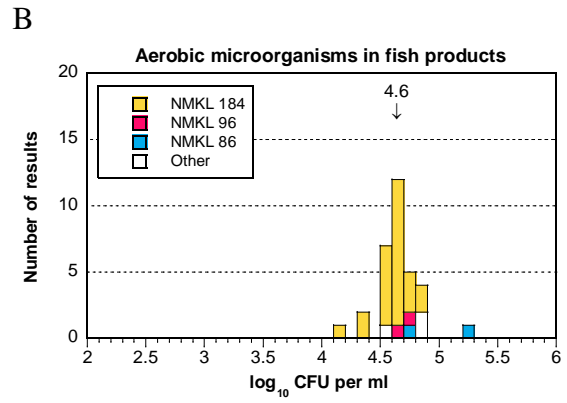
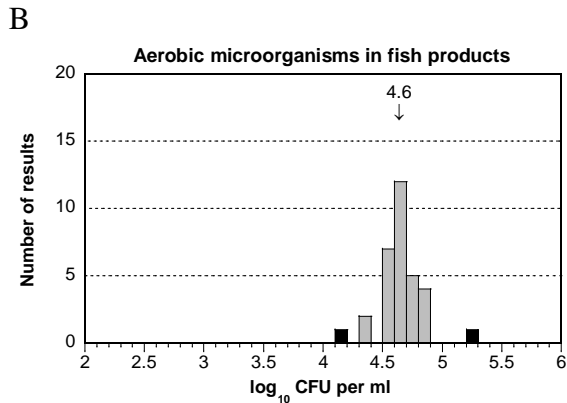
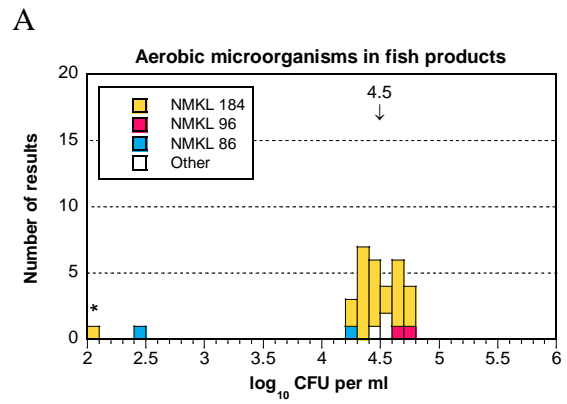
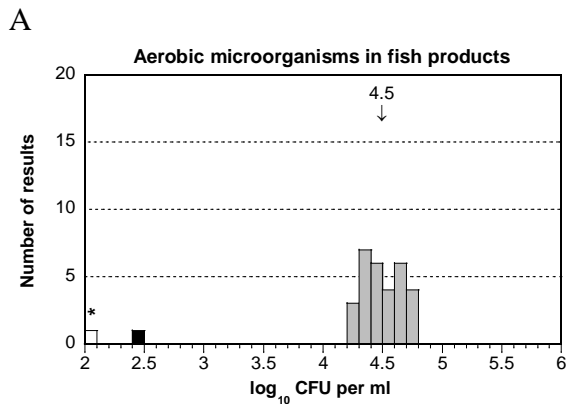
Most laboratories that performed both analyses, reported the use of iron agar and following NMKL 184:2006, which describes the determination of aerobic count and specific spoilage organisms in fish and fish products. Comparison with other methods and media (PCA and Petrifilm™) was difficult due to the low number of participating laboratories. It should nevertheless be mentioned that NMKL 86:2006 (Determination of Aerobic Microorganisms in Foods) and NMKL 96:2003 (Bacterial Examinations in Fresh and Frozen Seafood) have been replaced by NMKL 86:2013 and NMKL 96:2009

respectively – and that both of these refer to NMKL 184:2006 for the analysis of fish and fish products.

Results of aerobic microorganisms in fish products analysis

Method	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	32	30	4.49	0.16	1	1	0	30	4.64	0.12	0	1	1	30	4.48	0.21	0	2	0
NMKL 184:2006	25	24	4.48	0.17	1	0	0	24	4.62	0.12	0	1	0	23	4.44	0.17	0	2	0
NMKL 96:2003	2	2	4.67*	-	0	0	0	2	4.70*	-	0	0	0	2	4.59*	-	0	0	0
NMKL 86:2006	2	1	4.28*	-	0	1	0	1	4.72*	-	0	0	1	2	4.49*	-	0	0	0
Other	3	3	4.50*	-	0	0	0	3	4.80*	-	0	0	0	3	4.72*	-	0	0	0

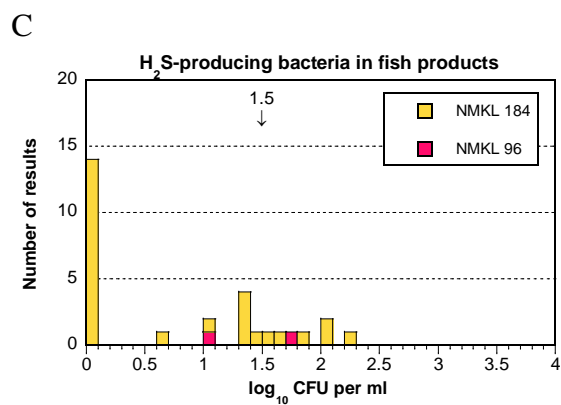
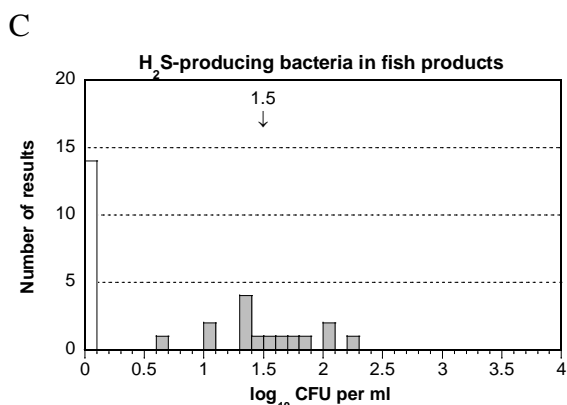
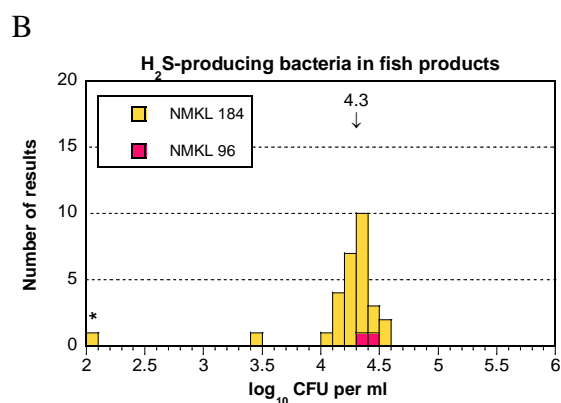
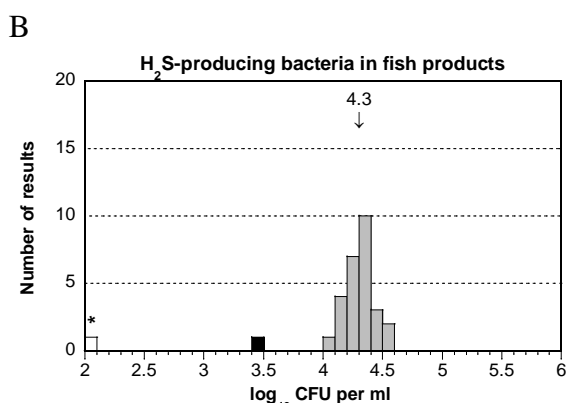
* Median



Results of H₂S producing bacteria in fish products analysis

Method	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	29	28	-	-	1	-	-	27	4.30	0.12	1	1	0	15	1.48*	-	14	0	0
NMKL 184:2006	27	26	-	-	1	-	-	25	4.29	0.12	1	1	0	13	1.48*	-	14	0	0
NMKL 96:2003	2	2	-	-	0	-	-	2	4.39*	-	0	0	0	2	1.38*	-	0	0	0
Other	0	0	-	-	0	-	-	0	-	-	0	0	0	0	-	-	0	0	0

* Median



Yeasts and moulds

Mixture A

A strain of *Candida glabrata* was target organism for the analysis of yeasts. The results were distributed with a primary peak around 2.4 log₁₀ CFU/ml, followed by a secondary small peak with high outliers around 4.0 log₁₀ CFU/ml. The latter were reported when using YGC, DG18/DRBC and DRBC, but were slightly more prevalent in the case of YGC. The only organism in the mixture present in a concentration corresponding to the outliers was *Staphylococcus saprophyticus*. This should normally not grow on the aforementioned media as long as antibiotics are present in the prescribed amounts. At the National Food Agency, no additional colonies except yeasts and moulds were observed on either DG18 or DRBC after 7 days incubation at 25 °C. For the analysis of

moulds, a strain of *Cladosporium cladosporioides* was target organism. False negative results were reported by 16 of 146 laboratories. Here as well, a small over-representation was found for YGC. However as the group of laboratories that reported false negatives for moulds only overlapped with two of the laboratories in the group that reported outliers for yeasts, a systematic error in the performance of the analyses can likely be ruled out.

Mixture B

Mixture B contained no target organism for the analysis of yeasts. Despite this, 8 of 145 laboratories reported false positive results. The reported concentrations ranged from 1.15 log₁₀ CFU/ml to 4.02 log₁₀ CFU/ml, and no correlation to method or media could be identified. A strain of *Penicillium verrucosum* was target organism for the analysis of moulds. The results were well-distributed, with a small number of outliers. Eight laboratories reported false negative results, but no correlation to method or media could be identified.

Mixture C

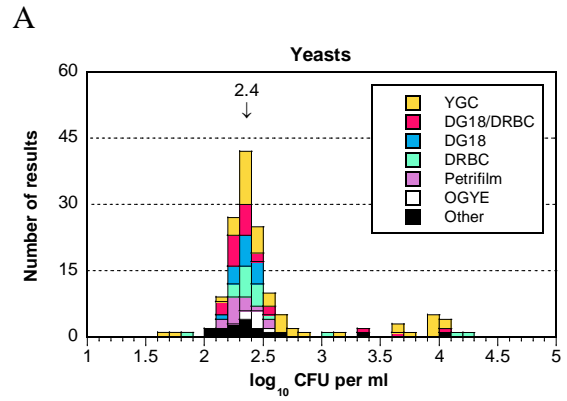
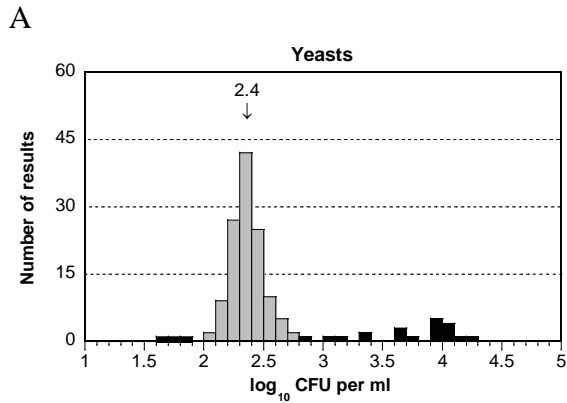
No target organism for either analysis was present in mixture C. The only deviating results were two false positives that were reported for moulds.

General remarks

The methods used were in principle identical for the analyses of yeasts and moulds, and consisted mainly of NMKL 98:2005, ISO 6611:2004/IDF 94:2004, and ISO 21527:2008. In mixture A, the use of YGC showed some correlation to high outliers for yeasts and false negatives for moulds. However in earlier PT rounds were similar mixtures have been used, no difference in the results were found when comparing YGC to other media. Finally, no media-attributed difference in the results could be found in mixtures B and C; meaning the variation observed for YGC is likely a random event.

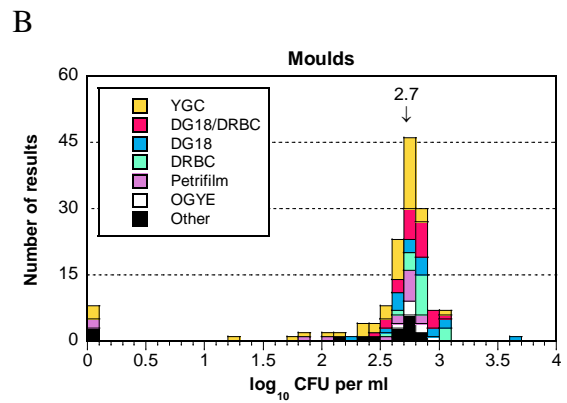
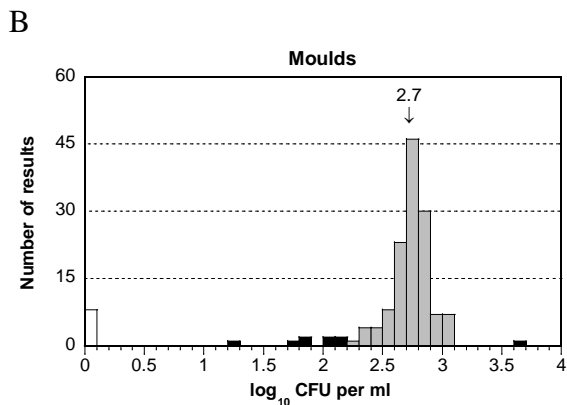
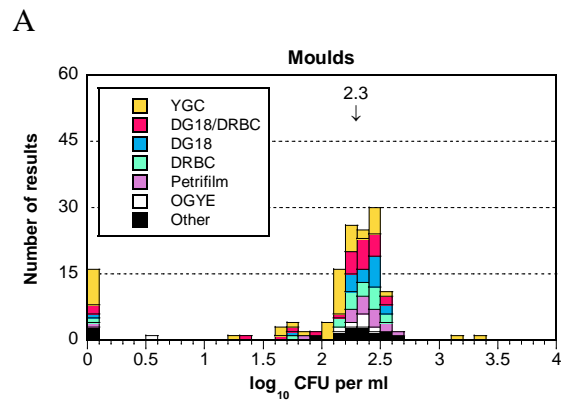
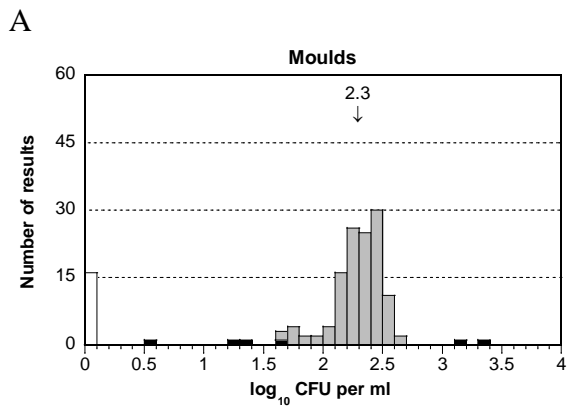
Results of yeasts analysis

Media	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	145	122	2.36	0.13	0	3	20	137	-	-	8	-	-	143	-	-	0	-	-
YGC	46	32	2.42	0.15	0	2	12	44	-	-	3	-	-	46	-	-	0	-	-
DG18/DRBC	24	21	2.30	0.11	0	0	3	24	-	-	0	-	-	24	-	-	0	-	-
DG18	17	17	2.33	0.09	0	0	0	16	-	-	1	-	-	16	-	-	0	-	-
DRBC	20	16	2.38	0.08	0	1	3	19	-	-	0	-	-	19	-	-	0	-	-
Petrifilm™	14	14	2.31	0.11	0	0	0	12	-	-	2	-	-	14	-	-	0	-	-
OGYE	7	7	2.42	0.06	0	0	0	7	-	-	0	-	-	7	-	-	0	-	-
Other	17	15	2.30	0.16	0	0	2	15	-	-	2	-	-	17	-	-	0	-	-



Results of moulds analysis

Media	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
Total	146	124	2.29	0.20	16	4	2	130	2.73	0.15	8	8	1	142	-	-	2	-	-
YGC	44	32	2.19	0.21	8	2	2	37	2.66	0.14	3	5	0	44	-	-	0	-	-
DG18/DRBC	26	23	2.29	0.22	2	1	0	26	2.77	0.14	0	0	0	25	-	-	0	-	-
DG18	18	17	2.34	0.18	1	0	0	17	2.75	0.20	0	0	1	17	-	-	1	-	-
DRBC	18	17	2.31	0.19	1	0	0	18	2.82	0.12	0	0	0	16	-	-	1	-	-
Petrifilm™	16	15	2.36	0.19	1	0	0	12	2.74	0.07	2	2	0	16	-	-	0	-	-
OGYE	7	6	2.33	0.11	0	1	0	7	2.79	0.08	0	0	0	7	-	-	0	-	-
Other	17	14	2.33	0.18	3	0	0	13	2.69	0.16	3	1	0	17	-	-	0	-	-



Outcome of the results of individual laboratory - assessment

These reported results of all participating laboratories are listed in Annex 1, together with the minimum and maximum accepted values for each analysis. Results that received a remark are also highlighted.

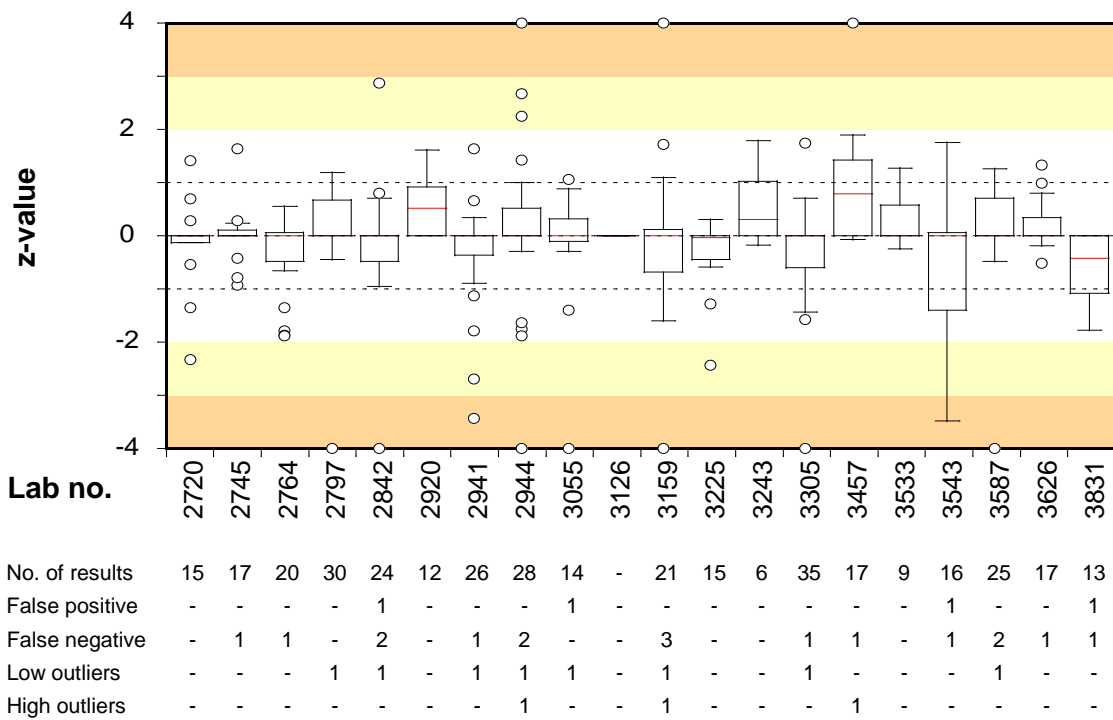
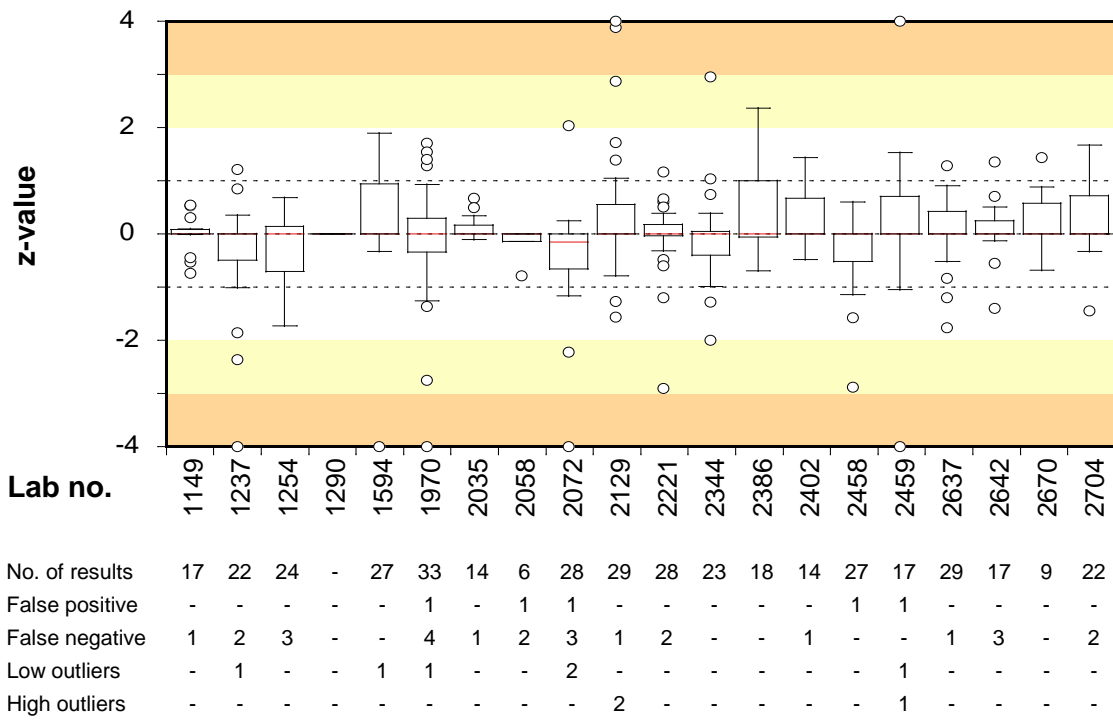
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated from all laboratory results for each analysis. The z-scores obtained, which are listed in Annex 2, can be used as a tool by laboratories when following up on the results.

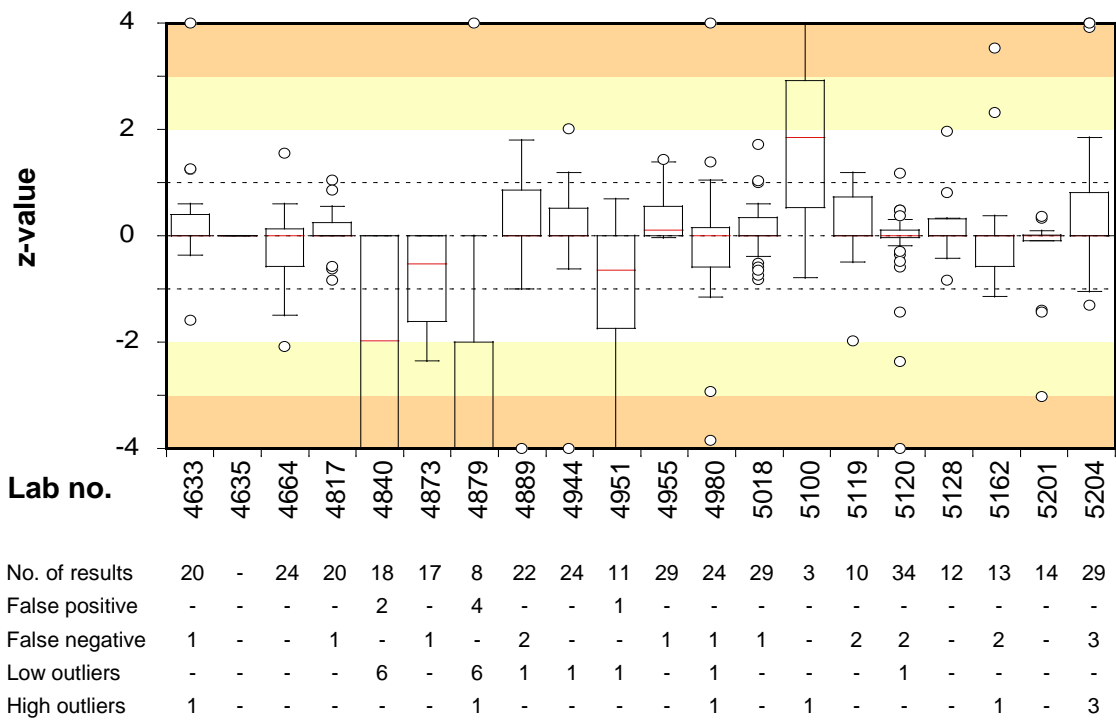
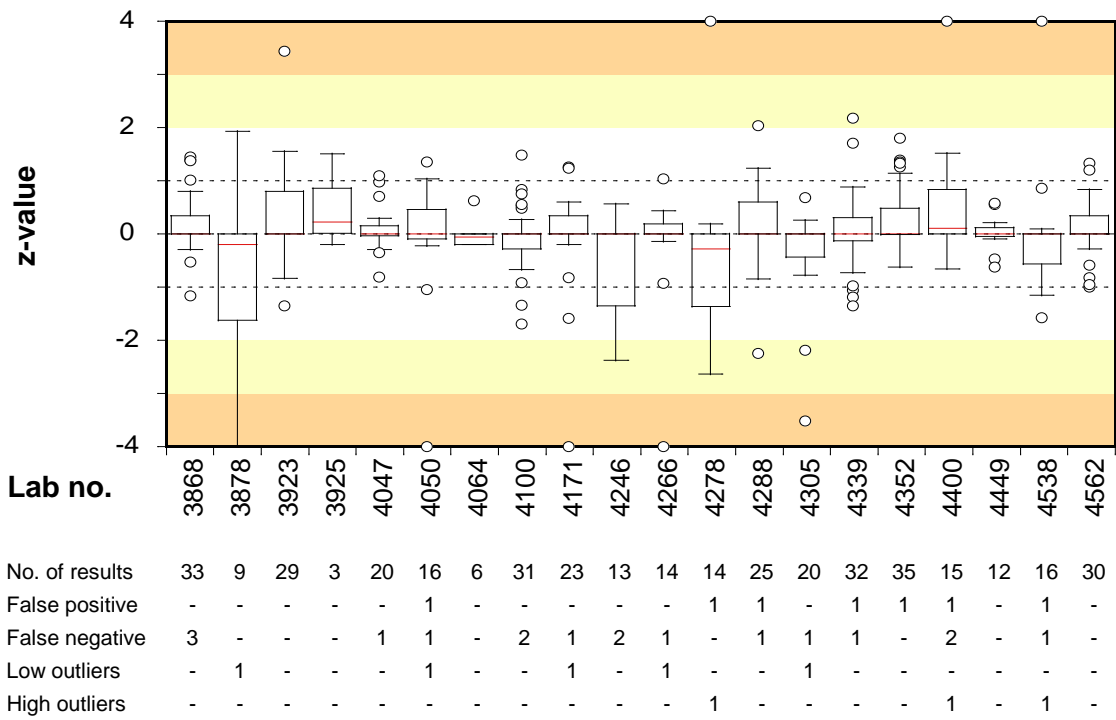
All results from each laboratory – outliers included and false results excluded – are compiled into a box plot based on their z-scores. A small box, centred around zero, indicates the results of that individual laboratory, with false results excluded, are close to the general mean values calculated for all laboratory results. The laboratories are 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.

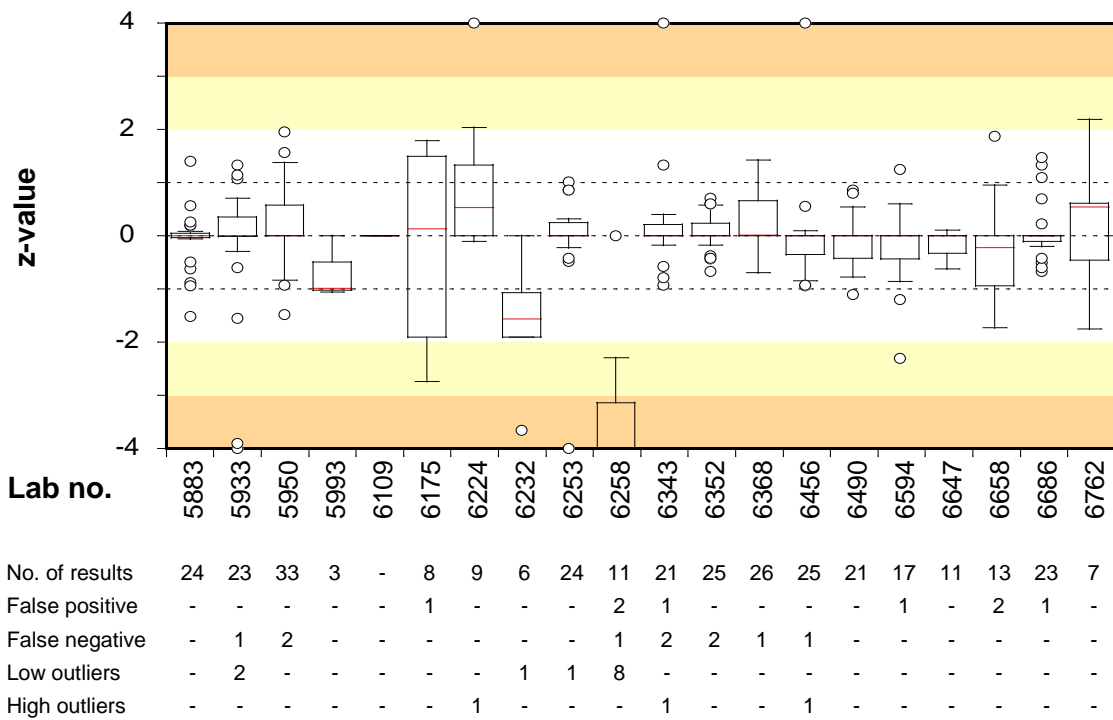
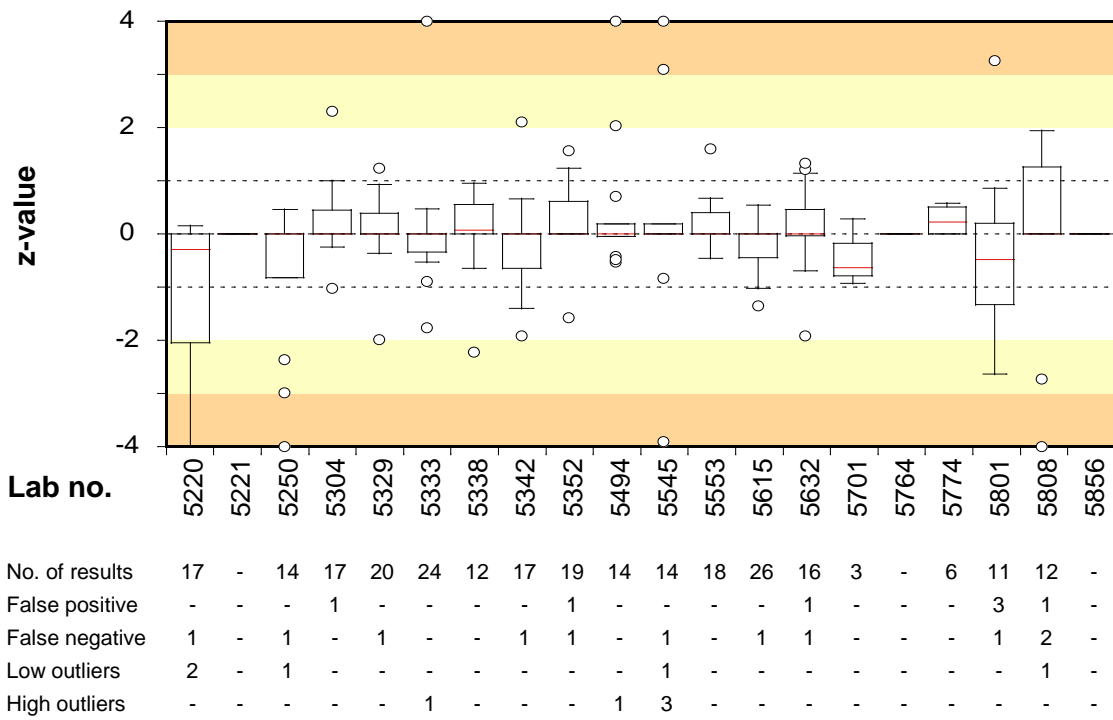
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (7). Samples for follow-up can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

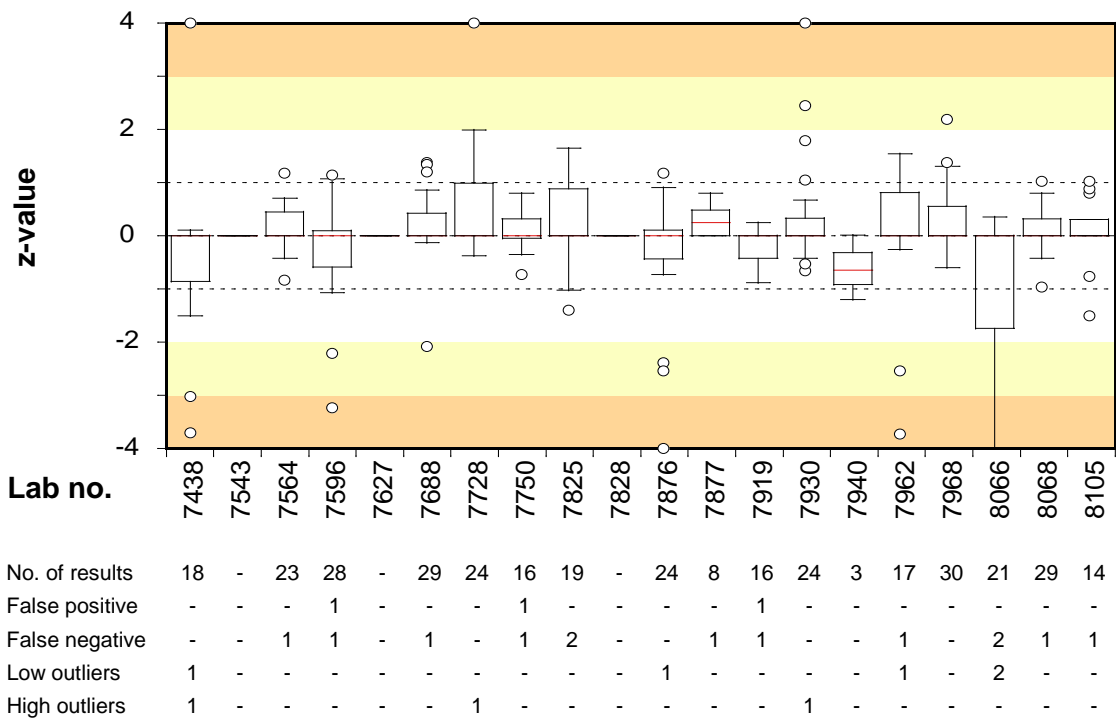
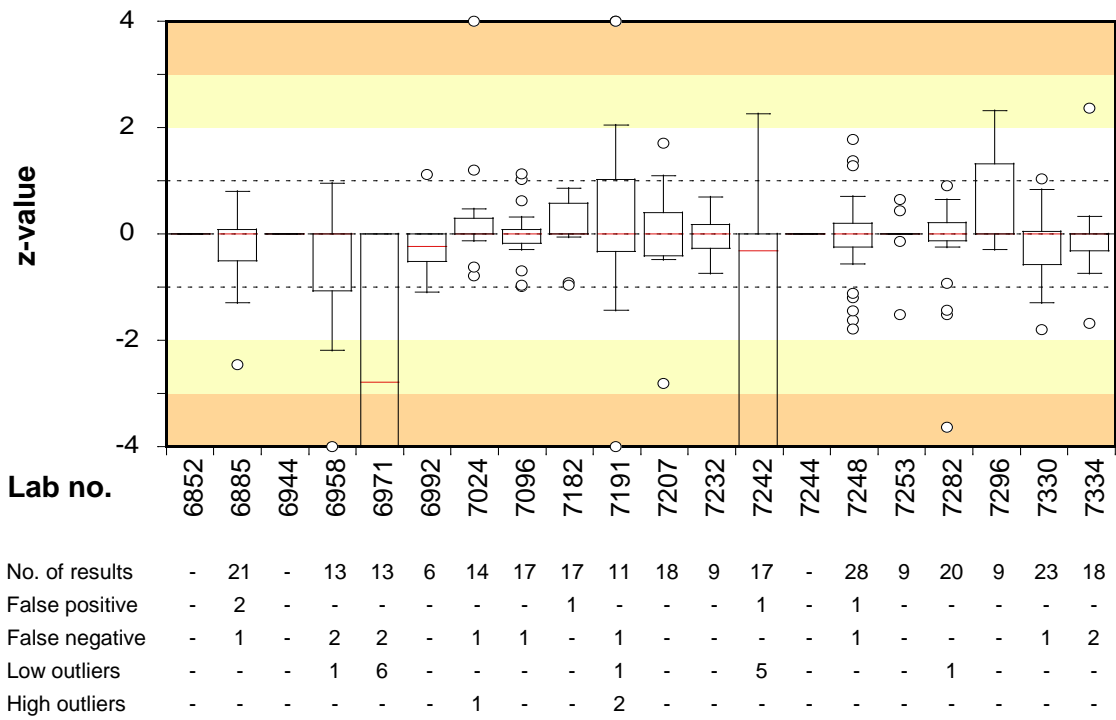
Box plots and numbers of deviating results for each laboratory

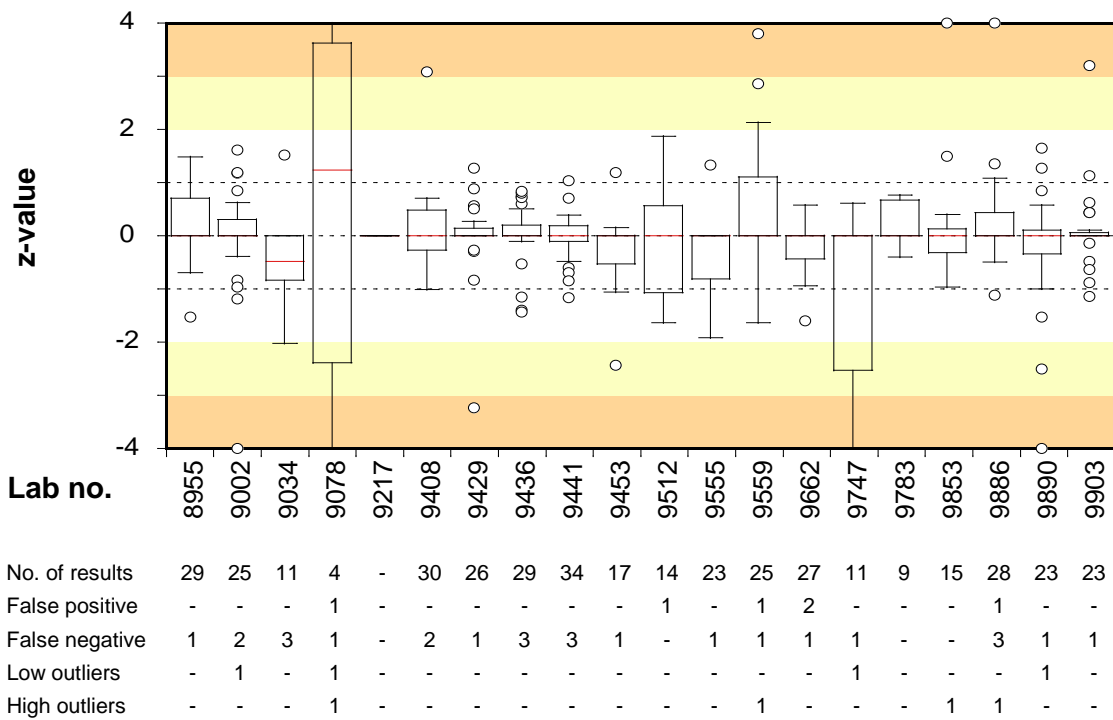
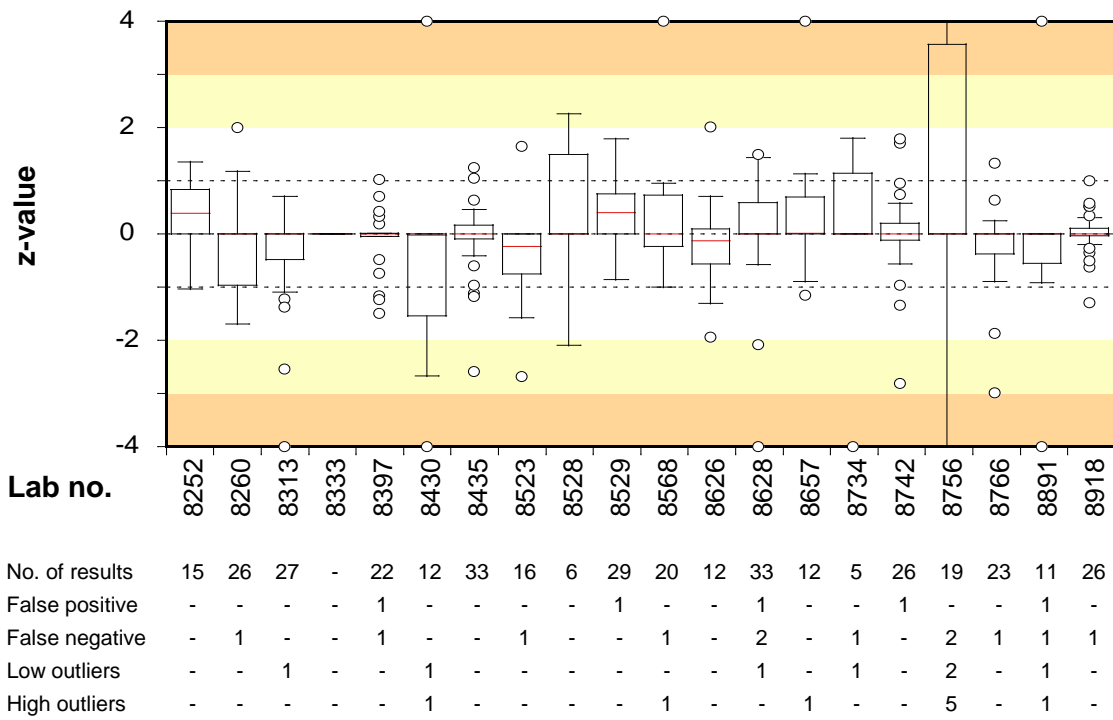
- *The plots are based on the laboratory results from all analyses transformed into z-scores calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation.*
- *Correct results for quantitative analyses without target organism generate a z-score of 0.*
- *The laboratory median value is illustrated by a horizontal red line in the box.*
- *The box includes 50 % of a laboratory's results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box.*
- *Very deviating results are represented by circles and are calculated as follow:
< [the lowest result in the box – 1.5 × (the highest result in the box – the lowest result in the box)]
or
> [the highest result in the box + 1.5 × (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.*

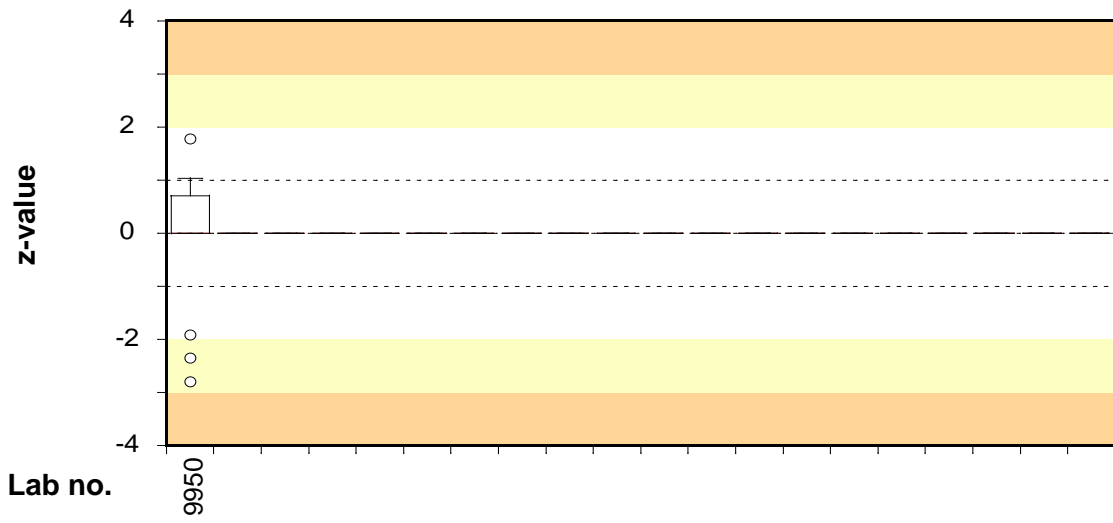












No. of results	13
False positive	-
False negative	2
Low outliers	-
High outliers	-

Test material and quality control

Test material

Each laboratory received three freeze-dried test items from 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 (8). 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>Pseudomonas aeruginosa</i>	SLV- 429
	<i>Staphylococcus aureus</i>	SLV- 280
	<i>Lactobacillus plantarum</i>	SLV- 475
	<i>Clostridium perfringens</i>	SLV- 442
	<i>Candida glabrata</i>	SLV- 052
	<i>Cladosporium cladosporioides</i>	SLV- 488
B	<i>Hafnia alvei</i>	SLV-015
	<i>Bacillus cereus</i>	SLV-517
	<i>Carnobacterium piscicola</i>	SLV-519
	<i>Clostridium bifermentans</i>	SLV-009
	<i>Penicillium verrucosum</i>	SLV-544
C	<i>Staphylococcus saprophyticus</i>	SLV- 013
	<i>Escherichia coli</i>	SLV- 295
	<i>Bacillus thuringiensis</i>	SLV- 564
	<i>Shewanella putrefaciens</i>	SLV- 520

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 (I₂) do not exceed simultaneously 2.6 and 2.0, respectively.

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

Analysis and method	A			B			C		
	<i>m</i>	<i>T</i>	<i>I</i> ₂	<i>m</i>	<i>T</i>	<i>I</i> ₂	<i>m</i>	<i>T</i>	<i>I</i> ₂
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.691	1.32	0.96	4.792	1.25	0.78	4.548	1.97	4.26
Psychrotrophic microorganisms NMKL method no. 86:2013	2.473	1.33	0.58	4.769	1.30	1.06	-	-	-
Enterobacteriaceae NMKL method no. 144:2005	-	-	-	4.313	1.59	5.62	3.190	1.17	0.50
<i>Escherichia coli</i> NMKL method no. 125:2005	-	-	-	-	-	-	3.143	1.25	0.92
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	-	-	-	3.575	1.32	0.741	3.295	1.44	0.65
Coagulase-positive staphylococci NMKL method no. 66:2009	3.897	1.33	1.63	-	-	-	-	-	-
Lactic acid bacteria NMKL method no. 140:2007	4.110	1.56	3.23	4.356	1.47	1.17	-	-	-
<i>Clostridium perfringens</i> NMKL method no. 95:2009	2.693	1.40	1.42	-	-	-	-	-	-
Anaerobic sulphite-reducing bacteria NMKL method no. 56:2008	2.754	1.37	1.33	2.304	1.90	2.02	-	-	-
Aerobic microorganisms in fish products NMKL method no. 184:2006	4.779	1.50	2.50	4.732	1.26	0.73	4.377	1.92	26.33
H ₂ S-producing bacteria in fish products NMKL method no 184:2006	-	-	-	4.462	1.29	0.48	1.783	1.63	0.421
Yeasts NMKL method no. 98:2005, DRBC	2.422	1.34	0.56	-	-	-	-	-	-
Moulds NMKL method no. 98:2005, DRBC	2.501	1.45	1.15	3.004	1.34	1.23	-	-	-

- No target organism or no value

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8. Peterz M, Steneryd, AC. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*, 74(2):143–148.

Lab no	Vial	Aerobic microorganisms 30 °C			Psychrotrophic microorganisms			Enterobacteriaceae			<i>Escherichia coli</i>			Presumptive <i>Bacillus cereus</i>			Coagulase-positive staphylococci			Lactic acid bacteria			<i>Clostridium perfringens</i>			Anaerobic sulphite-reducing bacteria			Aerobic m.o. in fish products, 20-25 °C			H ₂ S-prod. bacteria in fish products			Yeasts			Moulds			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				
4266	2 3 1	4.72	4.74	4.28	-	-	-	-	-	<1	<1	<1	-	-	-	2.78	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	<1	0.00	2.26	3	0	4266		
4278	2 3 1	4.41	4.59	4.15	-	-	-	<1	4.19	3.11	-	-	<1	3.27	2.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.79	2.29	<1	2	2.34	<1	4278			
4288	1 3 2	4.69	4.66	4.92	-	-	-	<1	4.15	3.04	<1	<1	3.08	<1	3.28	3.08	3.86	<1	<1	-	-	-	2.85	2.38	<1	2.23	<1	<1	-	-	-	-	-	-	2.49	<1	<1	2.51	3.04	<1	4288
4305	3 1 2	4.54	4.68	4.63	-	-	-	<1	4.16	3.08	-	-	-	<1	<1	2.7	3.79	<1	<1	-	-	-	-	-	-	1.95	2.6	<1	-	-	-	-	-	-	2.32	<1	<1	2.15	2.66	<1	4305
4339	3 1 2	4.6	4.83	4.34	-	-	-	<1	4.23	3.04	<1	<1	3.15	<1	<1	3.48	3.83	<1	<1	4	4.34	3.2	-	-	0	2.49	3.34	0	4.3	4.67	4.2	0	4.36	1.08	2.43	0	0	2.45	2.74	0	4339
4352	3 2 1	4.78	4.78	4.48	-	-	-	<1	4.45	3.15	<1	<1	3.04	<2	3.64	3.23	3.9	<1	<1	4.08	4.6	3.08	2.68	<1	<1	2.6	2.04	<1	4.78	4.6	4.48	<2	4.3	2	2.36	<1	<1	2.23	2.64	<1	4352
4400	3 1 2	4.61	4.81	4.36	-	-	-	<1	4.28	3.2	<1	<1	3.3	<1	3.51	3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.92	<1	<1	<1	<1	<1	4400	
4449	3 2 1	4.46	4.71	4.72	-	-	-	-	-	-	-	-	-	0	3.42	3.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.36	0	0	2.27	2.64	0	4449	
4538	2 3 1	4.69	4.67	4.47	-	-	-	<1	4.15	2.97	<1	<1	3.09	-	-	-	3.75	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	2.15	2.65	<1	3.33	<1	<1	4538		
4562	2 1 3	4.4	4.57	4.38	-	-	-	<1	4.38	3.23	<1	<1	3.08	<1	3.41	3.38	3.81	<1	<1	4.18	4.3	<1	2.66	<1	<1	2.66	1.79	<1	-	-	-	-	-	2.4	<1	<1	2.36	2.81	<1	4562	
4633	3 2 1	-	-	-	-	-	-	<1	<1	3.26	<1	<1	3.26	<1	3.3	2.82	3.79	<1	<1	-	-	-	2.85	<1	<1	3	<1	<1	-	-	-	-	-	4.05	<1	<1	2.41	2.7	<1	4633	
4635	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4635		
4664	1 2 3	4.81	4.76	4.59	-	-	-	<1	4.18	3.08	-	-	-	-	-	-	3.79	<1	<1	-	-	-	-	-	-	2.6	2.56	0	4.3	4.56	4.56	0	4.32	0.6	2.28	0	0	2.38	2.51	0	4664
4817	2 1 3	4.63	4.76	4.37	-	-	-	-	-	-	<1	<1	3.09	<1	<1	3.35	3.76	<1	<1	-	-	-	2.84	<2	<1	-	-	-	-	-	-	-	-	2.28	<1	<1	2.46	2.75	<1	4817	
4840	2 3 1	3.04	3.47	3.67	-	-	-	3.28	2.92	2.77	<1	<1	2.92	<1	2.82	2.25	3.04	<1	<1	3.11	2.89	3.77	2.34	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	4840		
4873	1 3 2	4.45	4.43	4.36	-	-	-	<1	4.45	3.02	-	-	-	<1	2.48	<1	-	-	-	-	-	-	-	-	-	-	2.37	1.13	<1	-	-	-	-	-	2.34	<1	<1	2.02	2.73	<1	4873
4879	1 3 2	2.41	1.56	2.34	-	-	-	1.46	1	2	1	0	1.23	-	-	-	7	1	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4879	
4889	2 3 1	4.72	4.79	4.43	-	-	-	<1	4.45	3.18	<1	<1	3.28	<1	<1	3.32	3.81	<1	<1	-	-	-	-	-	-	2.81	2.34	<1	4.36	4.52	3.52	<1	4.52	<1	-	-	-	-	-	4889	
4944	2 3 1	4.66	4.76	4.37	-	-	-	<1	4.54	3.04	<1	<1	3.01	<1	3.48	3.27	3.88	<1	<1	-	-	-	1.41	<1	<1	-	-	-	-	-	-	-	-	2.51	<1	<1	2.21	2.78	<1	4944	
4951	1 3 2	4.45	4.48	4.01	-	-	-	2.86	4.36	2.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.08	<1	<1	2.16	2.13	<1	4951	
4955	2 1 3	4.78	4.88	4.98	-	-	-	<1	4.45	3.18	<1	<1	3.13	<1	3.33	3.13	3.85	<1	<1	4.18	<2	<2	2.78	<0	<0	2.78	2.43	<0	-	-	-	-	-	2.37	<0	<0	2.4	2.75	<0	4955	
4980	2 1 3	4.38	4.79	4.32	-	-	-	<1	3.74	3.08	<1	<1	<1	<1	3.43	3.2	4.32	<1	<1	3.92	<2	<2	2	<1	<1	-	-	-	4.43	4.76	4.29	<1	4.47	1.48	-	-	-	-	-	4980	
5018	1 3 2	4.6	4.79	4.4	-	-	-	<1	4.32	3.05	<1	<1	2.99	<1	<1	3.34	3.83	<1	<1	4.32	3.79	<1	2.81	<1	<1	2.79	1.9	<1	-	-	-	-	-	2.4	<1	<1	2.16	2.89	<1	5018	
5100	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.07	>1.0	>1.0	2.13	3.01	>1.0	5100		
5119	2 3 1	4.2	4.64	4.77	-	-	-	-	-	-	<1	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.51	<1	<1	2.51	<1	<1	5119		
5120	2 1 3	4.48	4.75	4.38	-	-	-	<1	4.26	3.04	<1	<1	3.11	<1	<1	3.1	3.89	<1	<1	4.04	4.36	<2	2.73	<1	<1	2.74	2.5	<1	4.26	4.36	3.4	<1	4.36	<1	2.37	<1	<1	2.31	2.79	<1	5120
5128	1 3 2	4.88	4.82	4.43	-	-	-	-	-	-	<1	<1	3.08	<1	3.34	3.2	3.76	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5128	
5162	3 1 2	5.15	4.76	5.24	-	-	-	-	-	-	<1	<1	2.95	<1	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.28	<1	<1	2.06	2.61	<1	5162	
5201	3 1 2	-	4.71	4.14	-	-	-	-	4.31	3.1	-	<1	2.63	-	3.35	2.85	-	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	<1	<1	2.72	<1	5201		
5204	3 2 1	4.7	5.3	5.1	<1	4.4	2.7	<1	4.8	3	<1	<1	3.3	<1	3.5	3.2	3.8	<2	<2	3.9	4.6	<2	2.9	<1	<1	2.9	<1	<1	-	-	-	-	-	3.3	<1	<1	<1	2.6	<1	5204	
5220	3 2 1	4.19	4.3	4.39	-	-	-	0	4.1	2.85	0	0	1.9	0	0	1.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.28	0	0	2.32	2.69	0	5220	
5221	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5221		
5250	2 1 3	-	-	-	-	-	-	<1	4.28	2.78	<1	<1	2.95	<1	2.48	3.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.39	<1	<1	<1	1.81	<1	5250	
5304	1 2 3	4.57	4.77	4.25	-	-	-	-	-	-	<1	<1	3.04	-	-	-	3.86	<1	<1	-	-	-	2.9	2.85	<1	-	-	-	-	-	-	-	-	2.4	<1	<1	2.49	3.08	<1	5304	
5329	1 3 2	4.68	4.75	4.92	-	-	-	<1	4.25	3.14	-	-	-	<1	3.25	2.74	3.79	<1	<1	4.21	<2	<2	-	-	-	-	-	-	-	-	-	-	2.42	<1	<1	2.29	2.77	<1	5329		
5333	1 2 3	4																																							

Lab no	Sample	Aerobic microorganisms 30 °C			Psychrotrophic microorganisms			Enterobacteriaceae			Escherichia coli			Presumptive Bacillus cereus			Coagulase-positive staphylococci			Lactic acid bacteria			Clostridium perfringens			Anaerobic sulphite-reducing bacteria			Aerobic m.o. in fish products, 20-25 °C			H ₂ S-prod. bacteria in fish products			Yeasts			Moulds			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							
4100	3 1 2	-0.296	-0.200	-0.149				0	0.840	-0.481	0	0	-0.514	0	-0.916	-1.335	1.482	0	0	-0.008			0.538	0.276	0		-0.318	-0.674	0	0.484	0.755	-0.273	0	0	0.555	-1.697	0	4100			
4171	3 1 2	-0.818	-0.128	0.526				0	0.108	0.092	0	0	-4.000	0	0.456	1.255			-1.587	0		0.583	0.235	0				0	0.484	0.755	-0.196	0	0	0.605	1.241	0	4171				
4246	2 3 1	-1.050	-2.297	-1.331				0	-1.357	0.570	0	0				-2.373	0	0	-1.731	0																	4246				
4266	2 3 1	1.037	0.234	-0.926				0	0	0	0	0				-4.000	0	0																			4266				
4278	2 3 1	-0.760	-0.851	-1.365				0	-0.551	0.188			0	0.113	-2.231																						4278				
4288	1 3 2	0.863	-0.345	1.235				0	-0.844	-0.481	0	0	0.028	0	0.144	-0.289	0.711	0	0	0.595	0	-2.244															4288				
4305	3 1 2	-0.006	-0.200	0.256				0	-0.771	-0.099			0		-2.182		-0.368	0	0			-3.520	0.679	0													4305				
4339	3 1 2	0.342	0.884	-0.723				0	-0.258	-0.481	0	0	0.501	0		1.703	0.249	0	0	-0.582	0.160				-1.184	0.280	-1.355	0	0.484	-0.975	0.572	0	0	0.805	0.040	0	4339				
4352	3 2 1	1.385	0.523	-0.251				0	1.352	0.570	0	0	-0.243	0	1.266	0.458	1.328	0	0	-0.008	0.625				1.801	-0.318	0.008	0	-0.013	1.147	0.034	0	0	-0.292	-0.628	0	4352				
4400	3 1 2	0.400	0.740	-0.656				0	0.108	1.047	0	0	1.516	0	0.861	0.806																					4400				
4449	3 2 1	-0.470	0.017	0.560									0	0.580	0.209																						4449				
4538	2 3 1	0.863	-0.273	-0.284				0	-0.844	-1.149	0	0	0.095			-0.985	0	0																				4538			
4562	2 1 3	-0.818	-0.996	-0.588				0	0.840	1.334	0	0	0.028	0	0.549	1.205	-0.060	0	0	0.710	0.089	0	-0.191	0	0	-0.283	-0.956	0			0.341	0	0	0.356	0.507	0	4562				
4633	3 2 1							0	0	1.246	0	0	1.246	0	0.206	-1.584	-0.368	0	0			0.595	0	0	1.267													4633			
4635	1 3 2																																						4635		
4664	1 2 3	1.559	0.378	0.121				0	-0.625	-0.099						-0.368	0	0																					4664		
4817	2 1 3	0.516	0.378	-0.622									0	0.095		1.055	-0.831	0	0			0.554	0	0														4817			
4840	2 3 1	-4.000	-4.000	-2.986				-4.000	-3.059		0	0	-1.055	0	-1.290	-4.000	-4.000	0	0	-4.000	-2.434		-1.515	0														4840			
4873	1 3 2	-0.528	-2.008	-0.656				0	-1.723	-0.672			0	-2.350																									4873		
4879	1 3 2	-4.000	-4.000	-4.000				-4.000	-4.000		0	-4.000																												4879	
4889	2 3 1	1.037	0.595	-0.420				0	1.352	0.856	0	0	1.381	0	0.906	-0.060	0	0																					4889		
4944	2 3 1	0.690	0.378	-0.622				0	2.011	-0.481	0	0	-0.446	0	0.767	0.657	1.020	0	0			-4.000	0	0														4944			
4951	1 3 2	-0.528	-1.646	-1.838					0.693	-0.958																														4951	
4955	2 1 3	1.385	1.246	1.438				0	1.352	0.856	0	0	0.366	0	0.300	-0.040	0.557	0	0	0.710	0	0.306	0	0	0.264	0.336	0											4955			
4980	2 1 3	-0.934	0.595	-0.791				0	-3.846	-0.099	0	0		0	0.612	0.308	4.000	0	0	-1.156	0	-2.922	0																4980		
5018	1 3 2	0.342	0.595	-0.521				0	0.401	-0.385	0	0	-0.582	0	1.006	0.249	0	0	1.715	-0.824	0	0.430	0	0	0.310	-0.734	0											5018			
5100	3 1 2																																							5100	
5119	2 3 1	-1.978	-0.490	0.729																																				5119	
5120	2 1 3	-0.354	0.306	-0.588				0	-0.039	-0.481	0	0	0.231	0	-0.190	1.174	0	0	-0.295	0.196	0	0.099	0	0	0.082	0.477	0	-1.432	-2.365	-4.000	0	0.484						5120			
5128	1 3 2	1.965	0.812	-0.420									0	0.028	0	0.331	0.308	-0.831	0	0																			5128		
5162	3 1 2	3.530	0.378	2.316																																				5162	
5201	3 1 2		0.017	-1.399					0.327	0.092			-3.018		0.362	-1.435		0	0																				5201		
5204	3 2 1	0.921	4.000	1.843				-1.045	0	3.915	-0.863	0	0	1.516	0	0.830	0.308	-0.214	0	0	-1.300	0.625	0	0.802	0	0	0.811											5204			
5220	3 2 1	-2.041	-2.941	-0.558					0	-1.247	-2.324	0	0	-4.000		-4.000																								5220	
5221	3 2 1																																								5221
5250	2 1 3								0	0.093	-2.983	0	0	-0.825	0	-2.359	0.458																							5250	
5304	1 2 3	0.168	0.450	-1.027					0	0	-0.243																													5304	
5329	1 3 2	0.805	0.306	1.235					-0.112	0.474				0	0.050	-1.982	-0.368	0	0	0.925	0																		5329		
5333	1 2 3	-0.354	0.161	-0.420					0	-0.332	0.474	0	0	-0.040	0	0.113	-0.488	-0.523	0	0																			5333		
5338	1 2 3	-0.644	-2.225	0.155					0	0.840	0.474																													5338	
5342	1 3 2	-0.644	-1.140	-1.399					0	-0.771	0.665	0	0	0.366																									5342		
5352	3 2 1	0.632	0.595	0.256					0	1.572	0.856	0	0	0.840																										5352	
5494	1 3 2	-0.528	-0.417	-0.487					0	0.108	0.188				0	0.707																								5494	
5545	3 2 1								0	0.035	0.188				0	3.097	-0.831	0	0																					5545	
5553	2 1 3	0.168	-0.128	-0.453					0	0.401	0.092	0	0	0.028	0	0.487	1.603	0.557	0	0	0.678	0	0																5553		
5615	1 3 2	-0.180	-0.417	-0.048					0	-0.625	-0.481	0	0		0	-0.916	-0.289	-0.214	0	0	-1.018	0	0	0.173	0.538	0													5615		
5632	1 3 2	1.211	-0.056	0.661					0	-0.698	1.334				0	1.142	-0.190	-1.911	0	0																				5632	
5701	3 2 1	0.284	-0.634	-0.926																																					5701
5764	1 3 2																																								5764
5774	1 2 3																																								5774
5801	2 1 3	0.863	-1.213	-1.601					-0.917	-0.481					0.393	-1.435																									

Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most laboratories carry out some form of internal quality assurance, but their analytical work also has to be evaluated by an independent party. Such external quality control of laboratory competence is commonly required by accreditation bodies and can be done by taking part in proficiency testing (PT).

In a proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The organiser evaluates the results and compiles them in a report.

The National Food Agency's PT program offers

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

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

The National Food Agency's reference material

As a complement to the proficiency testing, National Food Agency produces also reference materials (RM) for internal quality control: a total of 8 RM for food and drinking water microbiological analyses, including pathogens, are available.

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