

## Food Microbiology

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April 2018



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Proficiency testing  
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
- H<sub>2</sub>S-producing bacteria in fish products
- Yeasts
- Moulds

## Abbreviations

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### Media

BA	Blood agar
BcsA	<i>Bacillus cereus</i> selective agar
BP	Baird-Parker agar
BP + RPF	Baird-Parker agar with rabbit plasma fibrinogen
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
CPC	CP ChromoSelect agar
DG18	Dichloran glycerol agar
DRBC	Dikloran Rose-Bengal chloramphenicol agar
EC	<i>E. coli</i> broth
EMB	Eosin methylene blue agar
IA	Iron agar
ISA	Iron sulphite agar
LTLSB	Lactose tryptone lauryl sulphate broth
mCP	Membrane <i>Clostridium perfringens</i> agar
MEA	Malt extract 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
MSA	Mannitol salt agar
MYP	Mannitol egg yolk polymyxin agar
OGYE	Oxytetracyclin glucose yeast extract agar
PAB	Perfringens agar base
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar
PCA	Plate count agar
PDA	Potato dextrose agar
RBC	Rose-Bengal chloramphenicol agar
SC	Sulphite cycloserine agar
SFP	Shahidi-Ferguson Perfringens agar
TBX	Tryptone bile X-glucuronide agar
TGE	Tryptone glucose extract agar
TS	Tryptose sulphite agar
TSA	Tryptone soya agar
TSC	Tryptose sulphite cycloserine agar
VRB	Violet red bile agar
VRBG	Violet red bile glucose agar
YGC	Yeast extract glucose chloramphenicol agar

### Organisations

AFNOR	French National Standardization Association
AOAC	AOAC INTERNATIONAL
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 after  $\log_{10}$  transformation were identified as statistical outliers (Grubbs' test modified by Kelly (1)). In some cases, subjective adjustments were made to set limits, based on knowledge of the mixture's contents. Outliers and false results were not included in the calculations of means and standard deviations. Results reported as "> value" were excluded from the evaluation. Results reported as "< value" were interpreted as being zero (negative result). All reported results are presented in Annex 1.

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



Mean values and standard deviations are normally provided for the different analyses. When the total number of reported results for an analysis is fewer than 20, the median is provided instead of the mean value. For method groups with fewer than 5 results, only the number of false results and outliers are provided. Results for such method groups are only displayed in tables and histograms when it is considered relevant.

### Uncertainty of measurement for the assigned values

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




### Table and figure legends

#### Tables

N	number of laboratories that performed the analysis
n	number of laboratories with satisfactory result
m	mean value in $\log_{10}$ cfu/ml (false results and outliers excluded)
s	standard deviation (false results and outliers excluded)
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 and parameter 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

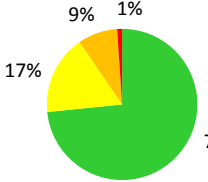
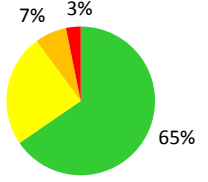
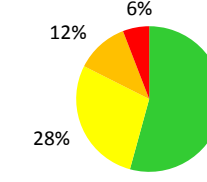
# Results of the PT round April 2018

## General outcome

Samples were sent to 195 laboratories, 44 in Sweden, 135 in other European countries, and 16 outside of Europe. Of the 188 laboratories that reported results, 117 (62 %) provided at least one result that received an annotation. In the previous round with similar analyses (April 2017), the proportion was 53 %.

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

**Table 1.** Composition of the test material and proportion of deviating results (N: number of reported results, F%: false positive or false negative, X%: outliers).

	Mixture A				Mixture B				Mixture C			
<b>% participants with</b>												
<b>Microorganisms</b>	<i>Escherichia coli</i> <i>Kluyveromyces marxianus</i> <i>Lactobacillus plantarum</i> <i>Penicillium verrucosum</i>				<i>Clostridium perfringens</i> <i>Hanseniaspora uvarum</i> <i>Serratia marcescens</i> <i>Shewanella putrefaciens</i> <i>Staphylococcus aureus</i>				<i>Bacillus cereus</i> <i>Carnobacterium piscicola</i> <i>Clostridium bifermentans</i> <i>Hafnia alvei</i> <i>Penicillium verrucosum</i>			
<b>Analysis</b>	<b>Target organism</b>	<b>N</b>	<b>F%</b>	<b>X%</b>	<b>Target organism</b>	<b>N</b>	<b>F%</b>	<b>X%</b>	<b>Target organism</b>	<b>N</b>	<b>F%</b>	<b>X%</b>
Aerobic micro-organisms, 30 °C	<i>L. plantarum</i> <i>E. coli</i>	167	0	4	<i>S. aureus</i> <i>S. putrefaciens</i>	166	1	6	<i>H. alvei</i> <i>C. piscicola</i>	166	1	4
Psychrotrophic microorganisms	<i>L. plantarum</i> <i>E. coli</i> <i>P. verrucosum</i>	16*	56*	0*	<i>S. aureus</i> <i>S. putrefaciens</i>	16	19	0	<i>H. alvei</i> <i>C. piscicola</i>	16	6	0
Enterobacteriaceae	<i>E. coli</i>	144	1	3	<i>S. marcescens</i>	144	5	4	<i>H. alvei</i>	144	2	6
<i>Escherichia coli</i>	<i>E. coli</i>	126	2	4	-	126	2	0	-	127	3	0
Presump. <i>B. cereus</i>	-	125	1	0	( <i>S. marcescens</i> )	125	6	0	<i>B. cereus</i>	126	6	5
Coagulase-positive staphylococci	-	113	0	0	<i>S. aureus</i>	113	5	6	-	113	1	0
Lactic acid bacteria	<i>L. plantarum</i>	67	0	6	( <i>S. aureus</i> )	68	29	0	<i>C. piscicola</i>	68	63	1
<i>C. perfringens</i>	-	63	0	0	<i>C. perfringens</i>	64	2	3	( <i>C. bifermentans</i> )	63	13	0
Anaerobic sulphite-reducing bacteria	-	73	3	0	<i>C. perfringens</i>	72	6	1	<i>C. bifermentans</i>	72	6	6
Aerobic microorg. in fish products	<i>L. plantarum</i> <i>E. coli</i>	35	0	3	<i>S. aureus</i> <i>S. putrefaciens</i>	35*	0*	0*	<i>H. alvei</i> <i>C. piscicola</i>	35	0	9
H <sub>2</sub> S-prod. bacteria i fish products	-	34	3	0	<i>S. putrefaciens</i>	34*	0*	0*	<i>H. alvei</i>	34	0	12
Yeasts	<i>K. marxianus</i>	149	12	3	<i>H. uvarum</i>	152	1	4	-	150	3	0
Moulds	<i>P. verrucosum</i>	150	6	7	-	149	4	0	<i>P. verrucosum</i>	152	7	7

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

\* the results are not evaluated

## Aerobic microorganisms, 30 °C

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### Mixture A

The strains of *L. plantarum* and *E. coli* were present in the highest concentrations and thus most colonies on the plates were from these species. Three low and three high outliers were reported.

### Mixture B

The strains of *S. putrefaciens* and *S. aureus* were present in the highest concentrations and thus most colonies were from these species. Six low and four high outliers were reported, as well as one false negative result. Slightly higher results were reported by users of 3M™ Petrifilm™ Aerobic Count (Petrifilm AC), something that has been observed also in previous proficiency testing rounds (latest in PT April 2017). The cause is unclear, but possibly the surface spreading technique used with Petrifilm AC is more gentle to the bacteria compared to the pour-plate technique used in other methods.

### Mixture C

The strains of *H. alvei* and *C. piscicola* were present in the highest concentrations and thus most colonies on the plates were from these species. Three low and three high outliers were reported, as well as one false negative result.

### General remarks

As a whole, the analyses were without problem for the laboratories. With the exception for slightly higher results for Petrifilm AC in mixture B, there was also no discernible difference between the different methods and media that were used.

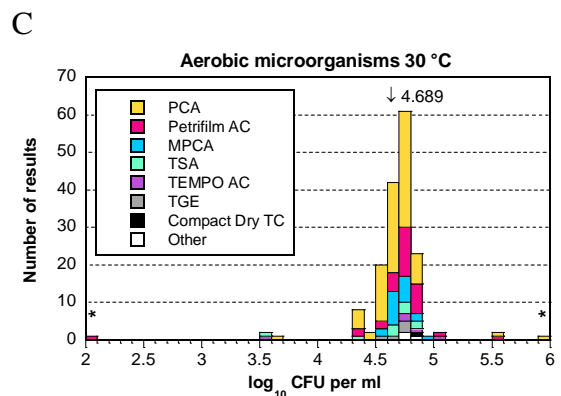
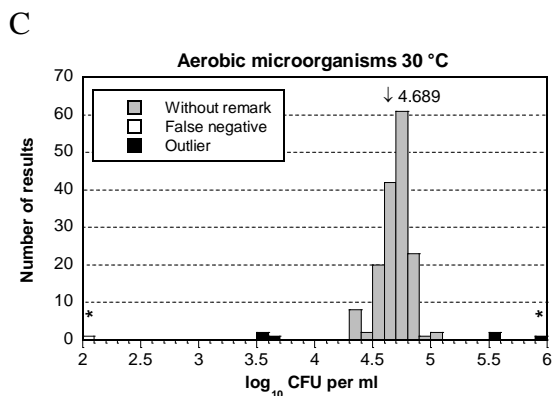
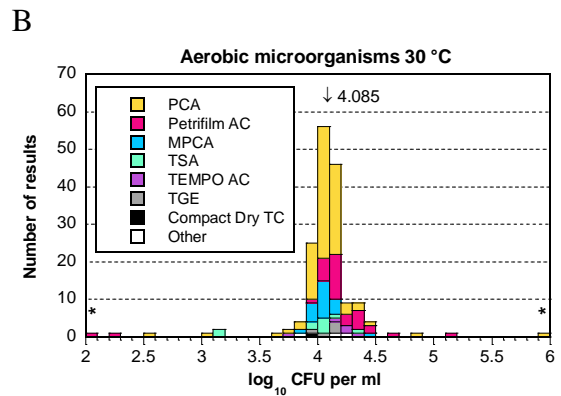
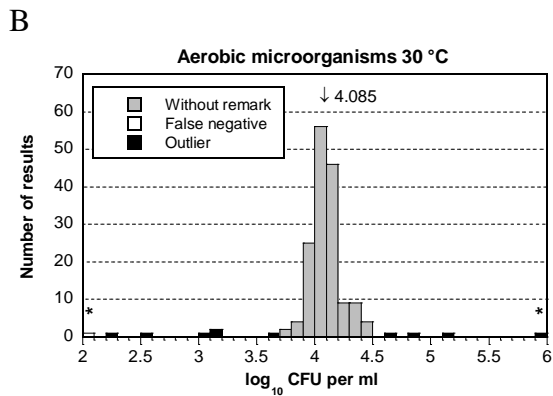
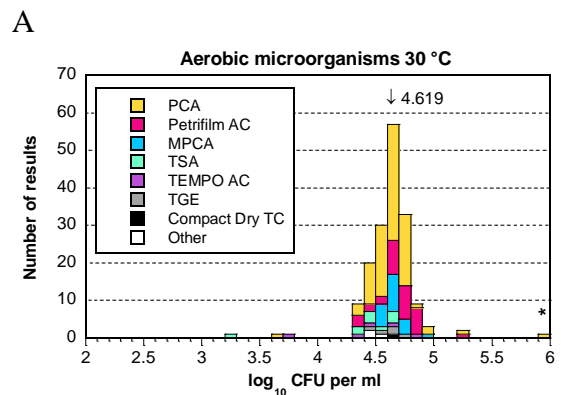
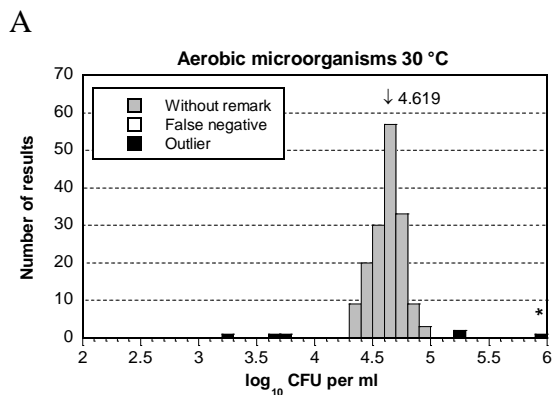
The majority of the laboratories followed either NMKL 86:2013 (29 %), ISO 4833-1:2013 (23 %) or used Petrifilm AC (19 %). The older NMKL 86:2006 was used by 10 % of the laboratories, while only a small number of laboratories stated the use of NMKL 86:2003 or ISO 4833:2003. These different methods are however highly similar, and all are based on incubation on Plate Count Agar (PCA) or Milk Plate Count Agar (MPCA) at 30 °C for 72 h. Users of Petrifilm AC may however use a different time/temperature, depending on which method is followed. For example, AOAC® 990.12 prescribes incubation at 35 °C for 48 h while AFNOR 3M 01/1-09/89 prescribes 30 °C for 72 h.

Five laboratories (3 %) used TEMPO® AC (bioMérieux® SA, Marcy l'Etoile, France), which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains wells with different volumes. A substrate in the wells emits fluorescence when hydrolysed by the microorganisms. The concentration is determined by the number and size of the fluorescent wells.



Results from analysis of aerobic microorganisms

Medium	N	Mixture A					Mixture B					Mixture C				
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >
All results	167	161	4.619	0.129	0	3 3	155	4.085	0.124	1	6 4	159	4.689	0.127	1	3 3
PCA	89	86	4.616	0.118	0	1 2	83	4.059	0.108	0	3 2	85	4.660	0.122	0	1 2
Petrifilm AC	33	32	4.661	0.153	0	0 1	29	4.177	0.123	1	1 2	31	4.727	0.144	1	0 1
MPCA	21	21	4.654	0.091	0	0 0	21	4.050	0.122	0	0 0	21	4.700	0.090	0	0 0
TSA	10	9	4.491	0.106	0	1 0	8	4.085	0.104	0	2 0	9	4.686	0.143	0	1 0
TEMPO AC	5	4	4.575	0.209	0	1 0	5	4.144	0.217	0	0 0	4	4.858	0.107	0	1 0
TGE	5	5	4.610	0.113	0	0 0	5	4.096	0.077	0	0 0	5	4.702	0.083	0	0 0
Compact Dry TC	1	1	-	-	0	0 0	1	-	-	0	0 0	1	-	-	0	0 0
Other	3	3	-	-	0	0 0	3	-	-	0	0 0	3	-	-	0	0 0



## Psychrotrophic microorganisms

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### Mixture A

The strains of *L. plantarum*, *E. coli* and *P. verrucosum* were target organisms. At the National Food Agency, a concentration of  $\log_{10}$  2.85 cfu ml<sup>-1</sup> was determined after ten days incubation on PCA at 6.5 °C. Nine of 16 laboratories reported zero results, in all cases except one after incubation at 6.5 °C or 7 °C. In comparison, the positive results were in all cases except one reported by laboratories that incubated at 15 °C or higher. The results for the seven laboratories that reported positive results varied between  $\log_{10}$  2.76-4.78 cfu ml<sup>-1</sup>, which can also partly be explained by the variations in the incubation conditions between the laboratories. The median for the positive results was 4.10 cfu ml<sup>-1</sup>, which is somewhat lower than the combined concentration of *L. plantarum* and *E. coli* in the mixture. Due to the differences in the incubation conditions, and the low number of participants, both zero results at incubation at 6.5/7 °C and positive results at 15 °C and higher are considered correct. The result for the laboratory that incubated at 20-21 °C, and still reported a negative result, is however considered incorrect.

*Comment: The results for mixture A are not statistically evaluated. Therefore, no z-scores have been calculated for these results, and they are also not included in the tables located below the box plots.*

### Mixture B

The strains of *S. aureus* and *S. putrefaciens* were target organisms. Three of 16 laboratories reported zero results. The 13 positive results were varied between  $\log_{10}$  2.90-4.39 cfu ml<sup>-1</sup>. The variation can here as well be partly explained by the variations in the incubation conditions between the laboratories. However contrary to the same analysis in mixture A, no obvious connection between reported results and incubation conditions could be identified. For example, a false negative result was reported by a laboratory that incubated at 20-21 °C, and high results were reported by laboratories that incubated at 6.5 °C. Combined with the low number of participants in the analysis, all positive results are considered correct.

### Mixture C

The strains of *H. alvei* and *C. piscicola* were target organisms. The majority of the laboratories also reported concentrations corresponding to the sum of *H. alvei* and *C. piscicola* in the mixture. One laboratory reported a false negative result. Due to the differences in the incubation conditions, and the low number of participants, all positive results are considered correct.

### General remarks

A total of 16 laboratories performed the analysis. The majority of these (81 %) incubated on PCA. The incubation conditions varied to a large extent, which is reflected by the differences in the methods that were used. NMKL 86:2013 prescribes 10 days at 6.5 °C, but 20 h at 17 °C followed by 3 days at 7 °C can also be used. For milk, ISO 6730:2005/IDF 101:2005 determines psychrotrophic microorganisms at 6.5 °C. The other method for milk, ISO 8552:2004/IDF 132:2004, instead estimates the number of psychrotrophic microorganisms in a rapid method based on incubation at 21 °C. ISO 4833-1:2013 prescribes incubation at 30 °C, but here the laboratories used both 6.5 °C and 20-21 °C.

Due to the low number of participants, the median is provided instead of the mean value in tables and figures.

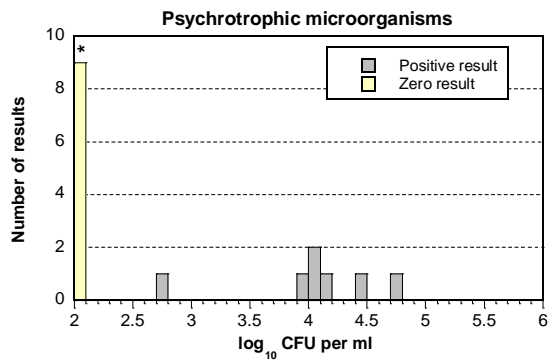
*Results from analysis of psychrotrophic microorganisms*

Temperature	N	Mixture A*					Mixture B					Mixture C				
		n	Med**	s	F	< >	n	Med**	s	F	< >	n	Med**	s	F	< >
All results	16	7	-	-	-	-	13	3.280	0.465	3	0 0 0	15	4.556	0.372	1	0 0 0
6.5 °C	6	1	-	-	-	-	5	3.340	0.562	1	0 0 0	5	4.663	0.097	1	0 0 0
20-21 °C	5	4	-	-	-	-	4	3.310	0.453	1	0 0 0	5	4.556	0.567	0	0 0 0
17 °C and 7 °C	4	1	-	-	-	-	3	-	-	1	0 0 0	4	-	-	0	0 0 0
15 °C	1	1	-	-	-	-	1	-	-	0	0 0 0	1	-	-	0	0 0 0

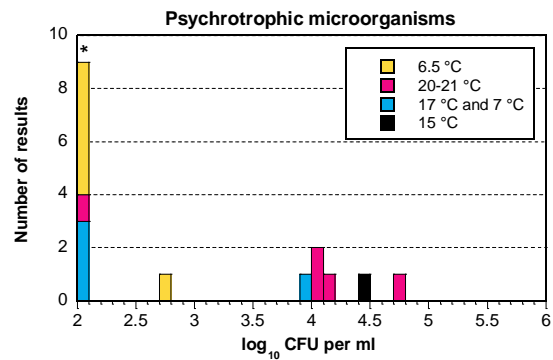
\* The results for mixture A are not evaluated

\*\* Med = median

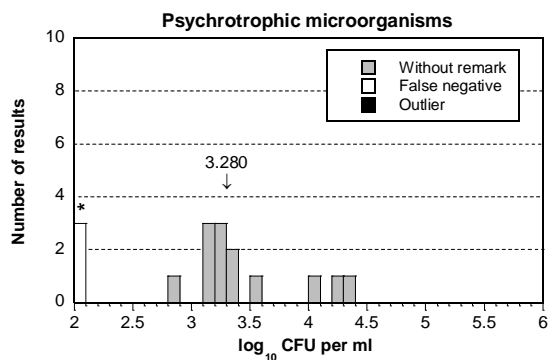
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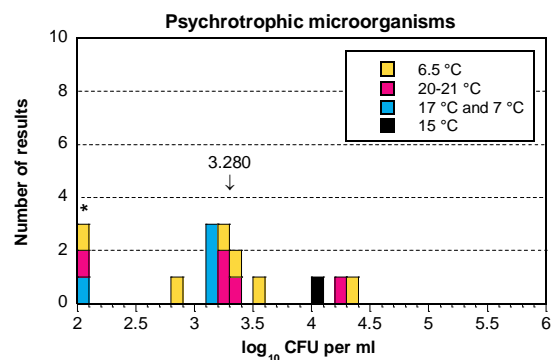
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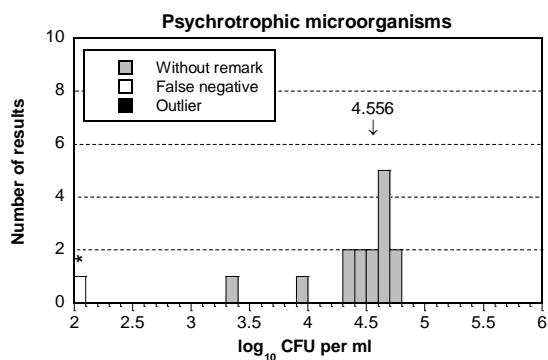
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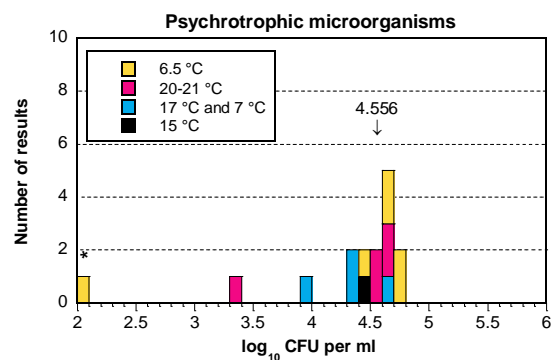
B



C



C



## Enterobacteriaceae

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### Mixture A

The strain of *E. coli* was target organism. Two low and two high outliers were reported, as well as one false negative result.

### Mixture B

The strain of *S. marcescens* was target organism. Three low and three high outliers were reported, as well as seven false negative results. Relatively few false negative results appear to have been reported by users of 3M™ Petrifilm™ Enterobacteriaceae (Petrifilm EB), but since the total number of false negative results is fairly low, it cannot be ruled out that this is simply due to chance.

### Mixture C

The strain of *H. alvei* was target organism. Six low and two high outliers were reported, as well as three false negative results. The majority of the low outliers were reported by users of violet red bile glucose agar (VRBG), which at the same time was the medium most frequently used.

### General remarks

As in previous proficiency testing rounds most laboratories used either NMKL 144:2005 (47 %) or a method based on Petrifilm EB (21 %), while the ISO methods (various versions) attributed to 20 %. The proportion of users of the new ISO 21528-2:2017 was now similar to the older ISO 21528-2:2004 (7 % and 8 % respectively). The new ISO 21528-1:2017 was however only used by two laboratories (1 %). ISO 21528-2:2017 is based on colony-count, whereas ISO 21528-1:2017 is based on MPN (Most Probable Number). The latter method is recommended when the expected level of Enterobacteriaceae is lower than 100 cfu g<sup>-1</sup>. The mean values for the different ISO methods were however very similar, for all three mixtures. As in the analysis of aerobic microorganisms, a small number of laboratories used fluorescence-based methods (TEMPO® Enterobacteriaceae). One laboratory reported following ISO 4832:2006, which is a method for enumeration of coliform bacteria at 30 °C or 37 °C.

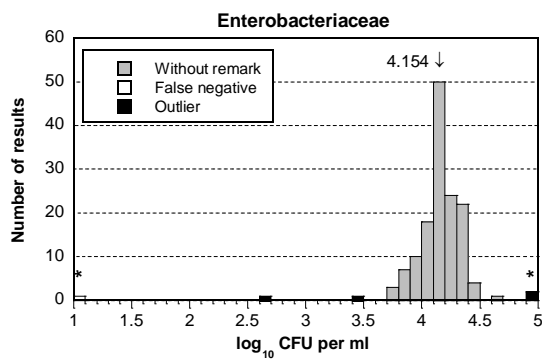
Enterobacteriaceae are Gram-negative and oxidase-negative bacteria that ferment glucose with the production of acid by-products. On VRBG – which is used in both NMKL 144 and ISO 21528-2 – they form pink/red colonies, with or without a bile precipitation zone. The appearance is similar on Petrifilm EB, which also includes a colour indicator for acid by-products and a plastic film for detection of gas production. According to NMKL 144:2005, presumptive colonies in VRBG should be confirmed with an oxidase test. In the new ISO 21528-2:2017, confirmation is instead done with a glucose oxidation/fermentation (OF) medium. Oxidase-negative bacteria that produce gas from glucose in the OF medium are considered as Enterobacteriaceae. The majority of the laboratories (65 %) stated performing some kind of confirmation test. No obvious difference could however be seen in the results from laboratories that performed such a test, and those that did not.

With the exception of the differences for VRBG and Petrifilm EB mentioned above, no major differences could be seen between the different methods and media that were used. It could still be mentioned that for TEMPO EB, slightly higher results could be seen in mixtures A and B, and slightly lower in mixture C, compared to other media. However TEMPO EB was only used by six laboratories and it is possible that these differences are simply due to chance.

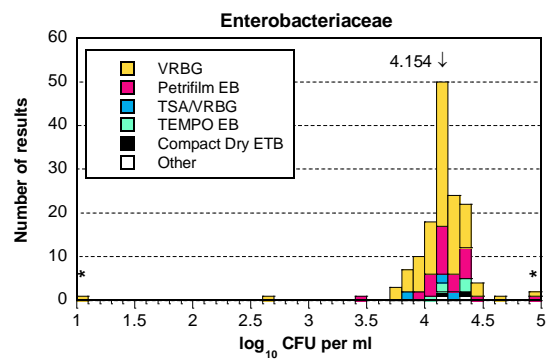
Results from analysis of Enterobacteriaceae

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
All results	144	139	4.154	0.152	1	2	2	131	2.651	0.167	7	3	3	133	4.204	0.163	3	6	2
VRBG	96	93	4.141	0.159	1	1	1	89	2.642	0.169	3	3	1	88	4.204	0.166	2	5	1
Petrifilm EB	32	30	4.180	0.124	0	1	1	26	2.657	0.139	4	0	2	30	4.225	0.152	1	0	1
TSA/VRBG	6	6	4.092	0.175	0	0	0	6	2.617	0.290	0	0	0	6	4.200	0.115	0	0	0
TEMPO EB	6	6	4.236	0.150	0	0	0	6	2.760	0.076	0	0	0	5	4.092	0.249	0	1	0
Compact Dry™ ETB	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0

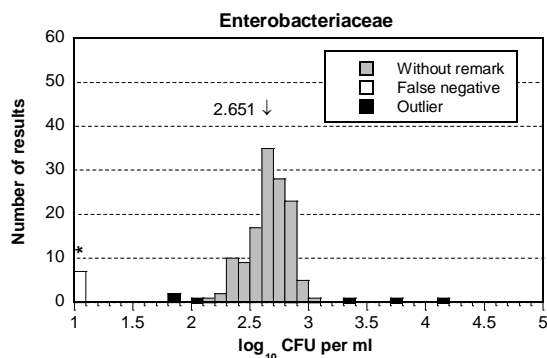
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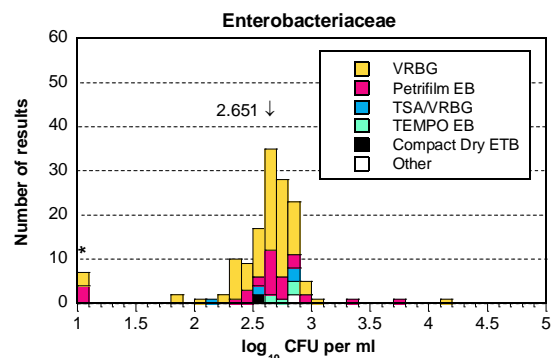
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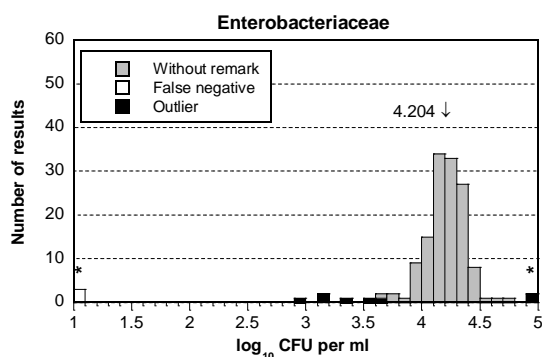
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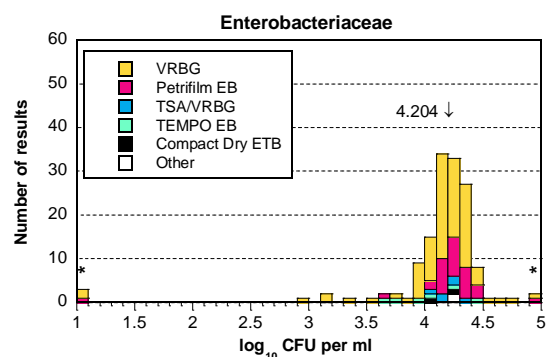
B



C



C



## *Escherichia coli*

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### **Mixture A**

The strain of *E. coli* was target organism. In tests at the National Food Agency, this was indole-positive,  $\beta$ -glucuronidase-positive, and formed gas in lactose tryptone lauryl sulphate broth (LTLBSB). Three low and two high outliers were reported, as well as three false negative results.

### **Mixture B**

No target organism was present in the mixture. Three laboratories reported a false negative result.

### **Mixture C**

No target organism was present in the mixture. Four laboratories reported a false negative result.

### **General remarks**

The use of 3M™ Petrifilm™ was high; in total 31 % of the laboratories used methods based on either Petrifilm EC/CC or Petrifilm SEC. These were followed by NMKL 125:2005 (29 %), and ISO 16649-2:2001 (13 %). Further, the MPN methods ISO 7251:2005 and NMKL 96:2009 were used by four and two laboratories, respectively. Two of the four laboratories that followed ISO 7251:2005 reported results that received an annotation. It is however difficult to determine if this is due to the strain being difficult with this particular method, or if it is simply due to chance.

The definition of *E. coli* differs between the methods. With ISO 16649-2:2001, *E. coli* are defined as those bacteria that form typical blue colonies on tryptone bile X-glucuronide agar (TBX) after 18-24 h at 44 °C. The blue colour is due to *E. coli*  $\beta$ -glucuronidase reacting with an indicator in the medium. No further confirmation of  $\beta$ -glucuronidase-positive colonies is required according to ISO 16649-2:2001. Petrifilm EC/CC and Petrifilm SEC are also based on media that detect *E. coli*  $\beta$ -glucuronidase. Further, the plastic film in Petrifilm EC/CC and Petrifilm SEC facilitates detection of gas produced by lactose fermentation. In comparison, NMKL 125:2005 describes the analysis of both thermotolerant coliform bacteria and of *E. coli*. Thermotolerant coliform bacteria are defined as those that form typical dark red colonies, surrounded by a red zone of precipitation, on violet red bile agar (VRB) after 24 h at 44 °C. Confirmation is by inoculation into either *E. coli* broth (EC) or LTLBSB. In both these media thermotolerant coliform bacteria produce gas, as a consequence of lactose fermentation. Bacteria that also produce indole in either LTLBSB or in tryptone broth are then considered as *E. coli*. In total, 59 % of the laboratories stated performing some kind of confirmation. No clear difference in the results of laboratories that confirmed, and those that did not, could however be discerned. Here, it could also be mentioned that NMKL 125 is currently being revised, and the new version will likely be more similar to ISO 16649-2.

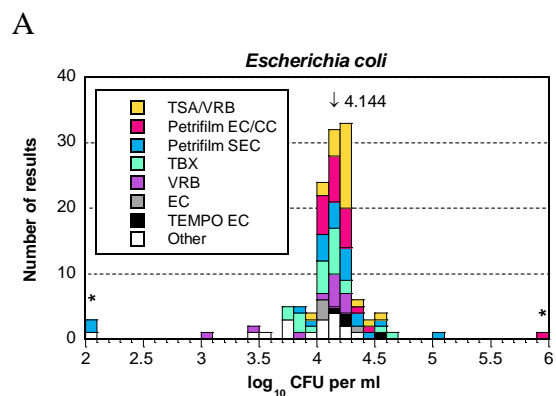
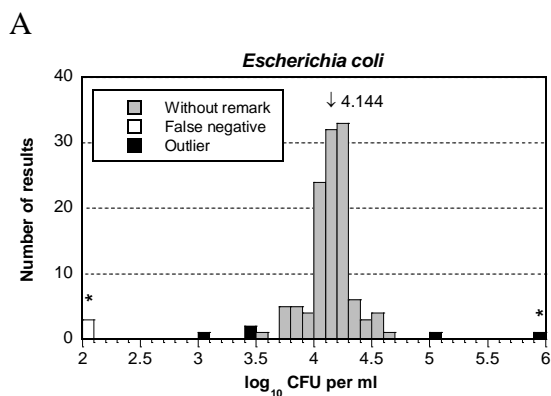
As in previous proficiency testing rounds where *E. coli* has been analysed, there were several methods and media that were only used by a small number of laboratories. Therefore, it was in general difficult to identify differences between methods and media. The exceptions to this were somewhat lower results for TBX, and somewhat higher for TSA/VRB. This has been observed in several previous proficiency testing rounds, but no clear cause has been found. It is however fairly likely that performing a pre-incubation may have an effect on the result. If the sample is suspected to contain

stressed microorganisms, ISO 16649-2:2001 prescribes a pre-incubation at 37 °C for 4 h prior to the final incubation at 44 °C. In comparison, a similar pre-incubation (20-25 °C for 1-2 h) is routinely carried out in NMKL 125:2005. Still, the differences were small, and the results also had a fairly wide distribution, at least for TBX.

The majority of the laboratories incubated either at 41.5-44 °C (63 %) or at 35-37 °C (36 %). Here it was obvious that for mixture A more outliers were reported by laboratories that incubated at the lower temperature (three low and two high outliers) compared to those that incubated at the higher temperature (no outliers). The number of false results also appeared to be somewhat over-represented for the 35-37 °C temperature range for mixtures B and C. The mean values for the two temperature groups were however similar.

### Results from analysis of *Escherichia coli*

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	126	118	4.144	0.172	3	3	2	123	-	-	3	-	-	123	-	-	4	-	-
TSA/VRB	23	23	4.213	0.120	0	0	0	23	-	-	0	-	-	23	-	-	0	-	-
Petrifilm EC/CC	22	21	4.163	0.095	0	0	1	19	-	-	1	-	-	20	-	-	1	-	-
Petrifilm SEC	22	19	4.174	0.164	2	0	1	22	-	-	0	-	-	21	-	-	1	-	-
TBX	22	22	4.088	0.211	0	0	0	22	-	-	0	-	-	22	-	-	0	-	-
VRB	12	10	4.137	0.103	0	2	0	11	-	-	1	-	-	11	-	-	1	-	-
EC	4	4	-	-	0	0	0	5	-	-	0	-	-	5	-	-	0	-	-
TEMPO EC	4	4	-	-	0	0	0	4	-	-	0	-	-	4	-	-	0	-	-
Rapid' E. coli 2	3	3	-	-	0	0	0	3	-	-	0	-	-	3	-	-	0	-	-
Brilliance EC/CC	2	1	-	-	0	1	0	2	-	-	0	-	-	2	-	-	0	-	-
CompactDry™ EC	2	2	-	-	0	0	0	2	-	-	0	-	-	1	-	-	1	-	-
EMB	2	0	-	-	1	0	0	2	-	-	0	-	-	2	-	-	0	-	-
TSA/VRBG	2	2	-	-	0	0	0	2	-	-	0	-	-	2	-	-	0	-	-
Other	7	7	3.986	0.203	0	0	0	6	-	-	1	-	-	7	-	-	0	-	-



## **Presumptive *Bacillus cereus***

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### **Mixture A**

No target organism was present in the mixture. One laboratory reported a false positive result.

### **Mixture B**

No target organism was present in the mixture. Seven laboratories reported a false positive result. The reported concentrations suggest they may have detected *S. aureus* or *S. marcescens*, which sometimes form atypical colonies on *Bacillus cereus*-selective agar (BcsA). At the National Food Agency, colonies were observed on blood agar (BA) – when transferred to BcsA they however displayed an atypical morphology and they did not have a blue colour.

### **Mixture C**

The strain of *B. cereus* was target organism. Two low and four high outliers were reported, as well as eight false negative results.

One laboratory detected a correct concentration (approximately 2100 cfu ml<sup>-1</sup>), but chose to report a negative result, since the colonies did not display a distinct blue colour on BcsA. The laboratory however made a comment that it could be a false negative result. No other laboratories have reported problems with the colour on BcsA.

### **General remarks**

As in previous proficiency testing rounds most laboratories followed either NMKL 67:2010 (53 %) or ISO 7932:2004 (26 %). These numbers indicate a slight increase in the use of the ISO method compared to the NMKL method. The remaining 22 % reported using either internal methods, company-specific methods or methods that were not further specified. ISO 7932:2004 was last reviewed in 2015 and remains current.

NMKL 67:2010 is based on growth on blood agar (BA). On this medium, *B. cereus* form large, irregular and grey colonies, that are surrounded by a large zone of haemolysis. Confirmation is by subculture onto either BcsA or Cereus-Ident agar (a chromogenic medium). On BcsA, presumptive *B. cereus* form bluish colonies that are surrounded by a precipitation zone due to lecithinase activity on egg yolk present in the medium. On Cereus-Ident agar, presumptive *B. cereus* are blue/turquoise, and possibly surrounded by a blue ring. The colour comes from enzymatic cleavage of the chromogenic substrate X-myoinositol-1-phosphate in Cereus-Ident agar, by *B. cereus* phosphatidylinositol phospholipase C (PI-PLC). In contrast to the NMKL method, ISO 7932:2004 prescribes the use of mannitol egg yolk polymyxin agar (MYP), which is followed by subculture onto BA. On MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a large zone of precipitation, again due to lecithinase activity. The confirmation consists of a positive result for haemolysis on BA.

As in previous proficiency testing rounds, the reporting of method data for *B. cereus* was in many cases unclear. For example, several laboratories reported using the same medium for both steps in the analysis. Other laboratories reported using combinations of methods and media that are incompatible. In general, the method/media reported by the laboratory are shown in this report, regardless if these are compatible or not. In addition to BA, BcsA and MYP, Oxoid Brilliance™ *Bacillus cereus* agar (CBC) – a chromogenic medium – was used by a group of eight laboratories. The substrate X-Gluc in CBC is cleaved by *B. cereus*  $\beta$ -glucuronidase, resulting in white colonies with a



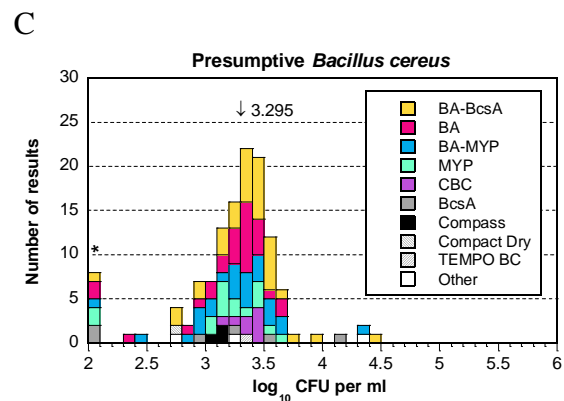
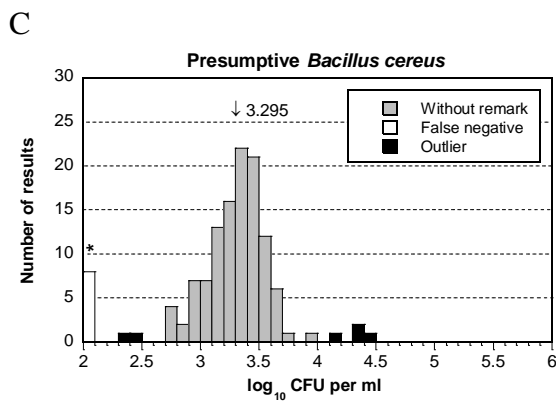
blue/green center. Laboratories that have only stated “chromogenic medium” for the analysis have been included in the group “Other”.

Despite the inconsistencies in the reporting of method data, the mean values for the different groups were highly similar. No differences in the results could be attributed to the use of a specific method or medium. Confirmation was performed by 63 % of the laboratories. No having performed a confirmation does not appear to have had an effect on the overall results of the laboratories. However, for mixture B somewhat more false positive results appear to have been reported by laboratories that did not perform a confirmation test, compared to those that did (five and two false positive results, respectively).

*Results from analysis of presumptive Bacillus cereus*

Medium	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	125	124	-	-	1	-	-	118	-	-	7	-	-	112	3.295	0.226	8	2	4
BA-BcsA*	34	34	-	-	0	-	-	31	-	-	2	-	-	32	3.347	0.253	1	0	1
BA	27	27	-	-	0	-	-	25	-	-	3	-	-	25	3.290	0.184	2	1	0
BA-MYP	25	25	-	-	0	-	-	26	-	-	0	-	-	23	3.284	0.236	1	1	1
MYP	17	16	-	-	1	-	-	15	-	-	1	-	-	14	3.283	0.182	2	0	0
CBC	8	8	-	-	0	-	-	8	-	-	0	-	-	8	3.378	0.118	0	0	0
BcsA*	6	6	-	-	0	-	-	6	-	-	0	-	-	3	3.255	0.303	2	0	1
Compass	3	3	-	-	0	-	-	3	-	-	0	-	-	3	-	-	0	0	0
Compact Dry BC	1	1	-	-	0	-	-	1	-	-	0	-	-	1	-	-	0	0	0
TEMPO BC	1	1	-	-	0	-	-	1	-	-	0	-	-	1	-	-	0	0	0
Other	3	3	-	-	0	-	-	2	-	-	1	-	-	2	-	-	0	0	1

\* Use of PEMBA has been interpreted as use of BcsA and is therefore included in this group.



## Coagulase-positive staphylococci

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### Mixture A

No target organism was present in the mixture. All laboratories reported a correct negative result.

### Mixture B

The strain of *S. aureus* was target organism. At the National Food Agency, this formed colonies on Baird-Parker agar with rabbit plasma fibrinogen (BP + RPF) and the analysis was unproblematic. The participating laboratories however reported five low and two high outliers, as well as six false negative results. Eleven of thirteen outliers or false negative results were from analyses with BP without RPF.

### Mixture C

No target organism was present in the mixture. One laboratory reported a false positive result.

### General remarks

As in previous proficiency testing rounds most laboratories (49 %) reported following NMKL 66:2009. Other methods used were either ISO 6888-1:1999 (13 %), 3M™ Petrifilm™ Staph Express (12 %) or ISO 6888-2:1999 (10 %). Both ISO 6888-1:1999 (based on BP) and ISO 6888-2:1999 (based on BP + RPF) were last reviewed by ISO in 2015 and remain current.

NMKL 66:2009 prescribes incubation on BP and/or BP + RPF. Blood agar (BA) may be used as a complement. On BP, *S. aureus* forms characteristic convex, shiny colonies that are grey/black due to reduction of tellurite in the medium. Proteolysis of egg yolk in the medium (due to lecithinase activity) normally causes a clear zone around the colonies. An opaque halo may also form near the colony, due to precipitation caused by lipase activity. The colonies are confirmed by a positive result in a coagulase test. When using BP + RPF, the coagulase activity is tested directly in the medium, and no subsequent confirmation is required. Similar to NMKL 66, ISO 6888-1 stipulates surface spreading on BP and confirmation by a coagulase test, whereas ISO 6888-2 instead uses BP + RPF. 3M™ Petrifilm™ Staph Express (Petrifilm Staph) uses a modified Baird-Parker medium, and a chromogenic indicator that stains *S. aureus* red/purple.

Coagulase-positive staphylococci are traditionally confirmed by detection of extracellular or bound coagulase (tube coagulase test and slide coagulase test respectively). Confirmation with latex agglutination test is also common. This is based on latex particles coated either with fibrinogen or with IgG that binds to protein A on the bacterial cell surface. Antibodies targeted against polysaccharides on the bacterial cell surface are also used in variations of this test. Users of Petrifilm Staph instead normally use 3M™ Petrifilm™ Staph Express Disk (Petrifilm Disk) for confirmation. This is based on detection of extracellular DNase, which is produced by the majority of coagulase-positive *S. aureus*, but also by *S. intermedius* och *S. hyicus*. Tolidine blue O in the disks visualizes DNase activity as a pink zone surrounding the colonies.

There is no clear explanation for the many outliers and false negative results in mixture B. Most of the outliers and false negative results were reported by users of BP, which at the same time was the most frequently used medium. At the same time, laboratories that incubated on BP + RPF, the second most frequently used medium,

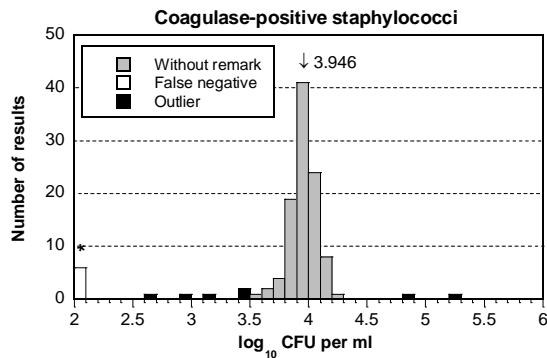
reported neither outliers nor false negative results for either of the mixtures. This could possibly suggest a problem with the confirmation among the laboratories that incubated on BP. Confirmation of some kind was performed by 73 % of the laboratories, and by 92 % of the laboratories that incubated on BP. The methods most used for the confirmation were tube coagulase test, latex agglutination test and Petrifilm Disk. Overall, laboratories that confirmed, and those that did not confirm, appear to have obtained similar results for all three mixtures. It could however be noted that none of the laboratories that reported outliers and false negative results for mixture B appear to have used a latex agglutination test.

*Results from analysis of coagulase-positive staphylococci*

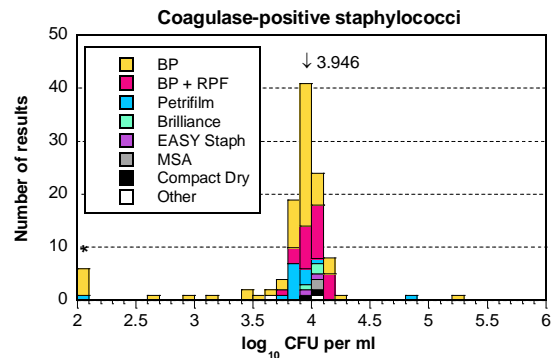
Medium	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	113	113	-	-	0	-	-	100	3.946	0.112	6	5	2	112	-	-	1	-	-
BP	62	62	-	-	0	-	-	50	3.938	0.113	5	5	1	62	-	-	0	-	-
BP + RPF	27	27	-	-	0	-	-	27	3.983	0.105	0	0	0	27	-	-	0	-	-
Petrifilm Staph	14	14	-	-	0	-	-	12	3.881	0.074	1	0	1	13	-	-	1	-	-
Oxoid Brilliance Staph 24	3	3	-	-	0	-	-	3	-	-	0	0	0	3	-	-	0	-	-
EASY Staph	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	-	-
MSA*	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	-	-
Compact Dry X-SA	1	1	-	-	0	-	-	2	-	-	0	0	0	1	-	-	0	-	-
Other	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	-	-

\* MSA: Mannitol salt agar

B



B



## Lactic acid bacteria

---

### Mixture A

The strain of *L. plantarum* was target organism. Four low outliers were reported.

### Mixture B

No target organism was present in the mixture. Nevertheless, 20 false positive results were reported. These are likely due to detection of *S. aureus*; strains of *S. aureus* have in previous proficiency testing rounds formed small colonies on de Man, Rogosa and Sharpe agar (MRS) and MRS with amphotericin (MRS-aB). At the National Food Agency, small transparent colonies were observed on MRS-aB at a concentration of  $\log_{10} 3.85 \text{ cfu ml}^{-1}$ . In the subsequent confirmation these were catalase positive, and they were therefore not considered as lactic acid bacteria. Thirteen of the 20 false positive results were from laboratories that did not perform a confirmation.

### Mixture C

The strain of *C. piscicola* was target organism. In total, 43 of the laboratories (63 %) reported a false negative result. The same mixture was used previously in the proficiency testing round April 2016, with a similar result (66 % false negative). Compared to other lactic acid bacteria, the strain of *C. piscicola* has a higher sensitivity to low pH, as for example in the media de Man, Rogosa and Sharpe-agar with sorbic acid (MRS-S) and Rogosa agar. Users of these media also often reported false negative results. Most false negative results were however reported by users of MRS. The remaining results were distributed around a distinct peak. One laboratory reported a low outlier.

### General remarks

The majority of the laboratories (56 %) followed NMKL 140. Most of them reported following NMKL 140:2007, but nine laboratories instead reported following the older NMKL 140:1991. This version prescribes spreading on MRS-S, while the newer version prescribes MRS-aB. On both media, lactic acid bacteria appear as 1.5-2 mm grey/white colonies. In comparison, ISO 15214:1998 uses a pour-plate method with MRS. This method was used by 15 % of the laboratories. It was last reviewed by ISO in 2015, but the review did not result in any changes. In comparison, NMKL 140 is scheduled for revision, and changes will likely be made to the confirmation tests.

Lactic acid bacteria constitute a heterogeneous group of microorganisms, and therefore have different optimal medium, pH and incubation conditions. For example MRS-aB (pH 6.2), which is used in NMKL 140:2007, is a less selective medium that allows the growth of a more wide range of lactic acid bacteria. This may however also result in the appearance of more false positive colonies compared to the more acid medium MRS-S (pH 5.7). Such differences between media and incubation conditions underline the importance of performing a confirmation test in uncertain cases, especially when using a less selective medium.

Both the ISO and the NMKL methods recommend confirmation of uncertain colonies by Gram staining and/or with a catalase test. Lactic acid bacteria are Gram positive and normally catalase negative. Confirmation was in this proficiency testing performed by 51 % of the laboratories; in most cases by a catalase test. Laboratories that reported false results for mixtures B and C do not appear to differ much in the performance or method of confirmation, compared to the other laboratories. For mixture

C however, the laboratories that performed a confirmation test had a higher mean value than the laboratories that did not perform a confirmation test ( $\log_{10}$  4.369 and  $\log_{10}$  4.031 cfu ml<sup>-1</sup> respectively).

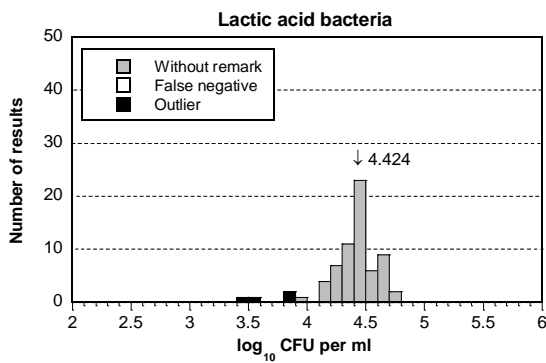
*Results from analysis of lactic acid bacteria*

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
All results	67	63	4.424	0.160	0	4	0	48	-	-	20	-	-	24	4.242	0.297	43	1	0
MRS	36	33	4.428	0.165	0	3	0	25	-	-	12	-	-	9	4.111	0.344	27	1	0
MRS-aB	12	12	4.437	0.169	0	0	0	10	-	-	2	-	-	10	4.399	0.248	2	0	0
MRS-S	8	8	4.390	0.107	0	0	0	4	-	-	4	-	-	4	4.180	0.180	4	0	0
Rogosa	7	6	4.493	0.140	0	1	0	7	-	-	0	-	-	0	-	-	7	0	0
Petrifilm LAB*	2	2	-	-	0	0	0	1	-	-	1	-	-	1	-	-	1	0	0
TEMPO LAB**	1	1	-	-	0	0	0	1	-	-	0	-	-	0	-	-	1	0	0
Other	1	1	-	-	0	0	0	0	-	-	1	-	-	0	-	-	1	0	0

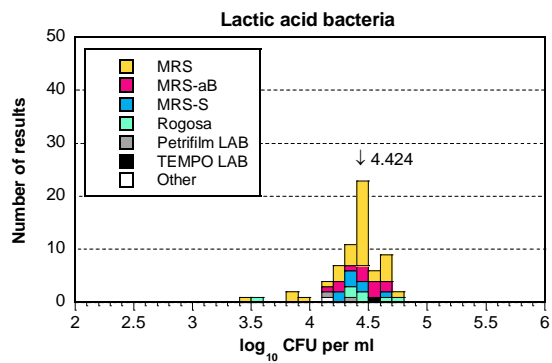
\* 3M™ Petrifilm™ Lactic Acid Bacteria Count Plate

\*\* TEMPO® Lactic Acid Bacteria

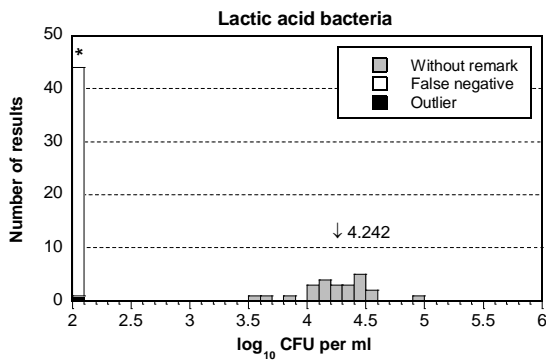
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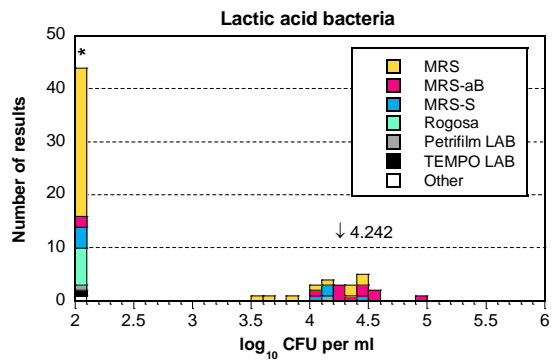
A



C



C



## *Clostridium perfringens*

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### **Mixture A**

No target organism was present in the mixture. All laboratories reported a correct negative result.

### **Mixture B**

The strain of *C. perfringens* was target organism. Two low outliers were reported, as well as one false negative result.

### **Mixture C**

No target organism was present in the mixture. However eight false positive results were reported. The concentrations of the false positive results corresponded to that of *C. bifermentans* in the mixture ( $\log_{10}$  2.494 cfu ml<sup>-1</sup>). At the National Food Agency, the strain of *C. bifermentans* formed black colonies on tryptose sulphite cycloserine agar (TSC). It could however be distinguished from *C. perfringens* in subsequent confirmation, since the strain was motile and did not ferment lactose. Five of the eight laboratories that reported a false negative result stated they performed some kind of confirmation.

### **General remarks**

As in previous proficiency testing rounds most laboratories followed either NMKL 95:2009 (63 %) or ISO 7937:2004 (25 %). Two and one laboratory followed the older NMKL 95:2006 and NMKL 95:1997, respectively. Two other laboratories stated following NMKL 56 (Sulphite-reducing Clostridia). This method includes detection of *C. perfringens* by referring to the confirmation tests in NMKL 95. ISO 7937:2004 was last reviewed by ISO in 2015 and remains current. It is however scheduled to be replaced by ISO 15213-2 ("Enumeration of *Clostridium perfringens* by colony-count technique"), which is currently under development. No differences could be seen in the results from the different methods that were used.

ISO 7937:2004 prescribes a pour-plate method with TSC, while NMKL 95 prescribes surface-spreading on mCP and/or pour-plating with TSC. The majority of the laboratories (89 %) reported using TSC. On TSC, *C. perfringens* form black colonies after anaerobic incubation at 37 °C. Suspected and typical colonies are in NMKL 95:2009 confirmed with a motility test and a test for lactose fermentation. *C. perfringens* are non-motile and form acid and gas as a consequence of lactose fermentation. The method for confirmation is similar in ISO 7937:2004. In total, 92 % of the laboratories stated performing some kind of confirmation. The low number of laboratories that did not confirm makes it difficult to determine if this had an effect on the overall result. Similar results do however appear to have been reported regardless if a confirmation was performed or not.

Two of the laboratories that followed NMKL 95:2009 incubated on mCP. This medium was previously used in some countries for membrane filter analyses of drinking water. In such analyses, mCP has sometimes been found to result in lower recovery of *C. perfringens* compared to TSC (2, 3, 4). Comparative studies on food analyses have also advocated the use of TSC as the preferred medium for detecting *C. perfringens* (5, 6). The results for mCP – but also for sulphite cycloserine agar (SC) – also appear to be somewhat lower than the overall mean value. These media were at the same time used

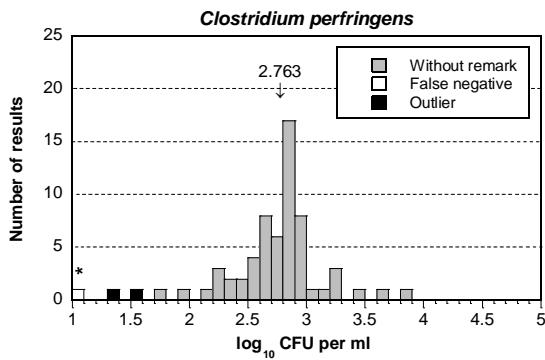
by only two laboratories each, which makes it difficult to come to a definite conclusion based on this observation.

The majority of the laboratories (92 %) incubated at 37 °C. The remaining five laboratories incubated at 44 °C. The low number of laboratories that incubated at 44 °C makes it difficult to draw conclusions on the effect of the incubation temperature on the outcome – especially since three of the five laboratories did not perform a confirmation.

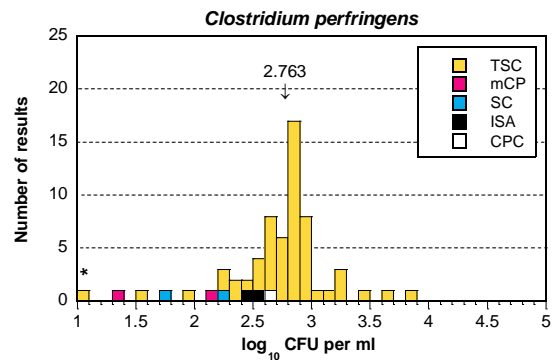
*Results from analysis of Clostridium perfringens*

Method	N	Mixture A						Mixture B						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	63	63	-	-	0	-	-	61	2.763	0.349	1	2	0	55	-	-	8	-	-
TSC	56	56	-	-	0	-	-	55	2.812	0.318	1	1	0	48	-	-	8	-	-
mCP	2	2	-	-	0	-	-	1	-	-	0	1	0	2	-	-	0	-	-
SC	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	-	-
ISA	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	-	-
CPC	1	1	-	-	0	-	-	1	-	-	0	0	0	1	-	-	0	-	-

B



B



## **Anaerobic sulphite-reducing bacteria**

---

### **Mixture A**

No target organism was present in the mixture. Two laboratories reported a false positive result.

### **Mixture B**

The strain of *C. perfringens* was target organism. One high outlier was reported, as well as four false negative results.

### **Mixture C**

The strain of *C. bifermentans* was target organism. Two low and two high outliers were reported, as well as four false negative results.

### **General remarks**

As in previous proficiency testing rounds most laboratories followed a version of NMKL 56. However only a few laboratories (10 %) reported following the new NMKL 56:2015. Instead, most still followed NMKL 56:2008 (52 %) or the significantly older NMKL 56:1994 (4 %). In comparison, ISO 15213:2003 was followed by 15 % of the laboratories. This was last reviewed by ISO in 2015 and remains current. It is however scheduled to be replaced by ISO 15213-1 ("Enumeration of sulphite-reducing *Clostridium* spp. by colony-count technique"), which is currently under development. Two laboratories followed ISO 7937:2004 ("Horizontal method for the enumeration of *Clostridium perfringens*"), which will be replaced by the future ISO 15213-2 ("Enumeration of *Clostridium perfringens* by colony-count technique"). Regardless, no differences could be seen in the results from the different methods that were used.

Both NMKL 56:2015 and ISO 15213:2003 prescribe pour-plate methods with iron sulphite agar (ISA). ISA was also the medium most frequently used by the laboratories. Black colonies on ISA (possibly surrounded by a black zone) are considered as sulphite-reducing bacteria. The black colour of the colonies comes from iron sulphide, which is formed as a precipitate of  $\text{Fe}^{3+}$  in the medium and  $\text{H}_2\text{S}$  that is produced by the reduction of sulphite. Growth of anaerobic bacteria that only produce hydrogen (and not  $\text{H}_2\text{S}$ ) may sometimes result in a diffuse and unspecific blackening of the medium.

In addition to ISA, laboratories also reported using tryptose sulphite cycloserine agar (TSC), Shahidi-Ferguson Perfringens agar (SFP), Perfringens agar base (PAB) and tryptose sulphite agar (TS). These media are often used when identifying *C. perfringens*, and it should therefore be mentioned that for that purpose, colonies should be confirmed using the methods in for example NMKL 95. There was however no apparent difference in the results for laboratories that used these media.

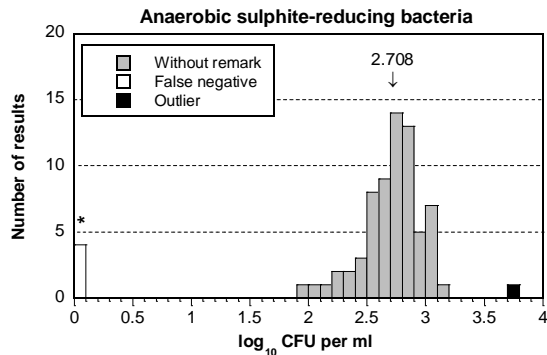


Results from analysis of anaerobic sulphite-reducing bacteria.

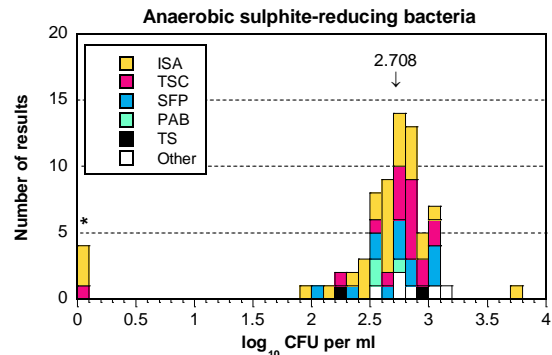
Method	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	73	71	-	-	2	-	-	67	2.708	0.246	4	0	1	64	2.314	0.310	4	2	2
ISA	31	31	-	-	0	-	-	26	2.632	0.242	3	0	1	27	2.310	0.335	2	1	0
TSC	18	17	-	-	1	-	-	17	2.796	0.196	1	0	0	17	2.354	0.279	0	0	1
SFP	13	13	-	-	0	-	-	13	2.718	0.293	0	0	0	10	2.238	0.349	1	1	1
PAB	3	3	-	-	0	-	-	3	-	-	0	0	0	3	-	-	0	0	0
TS	2	2	-	-	0	-	-	2	-	-	0	0	0	2	-	-	0	0	0
Other*	6	5	-	-	1	-	-	6	2.841	0.208	0	0	0	5	2.225	0.335	1	0	0

\* The group Other mainly includes laboratories with unclear or ambiguous reporting of the medium.

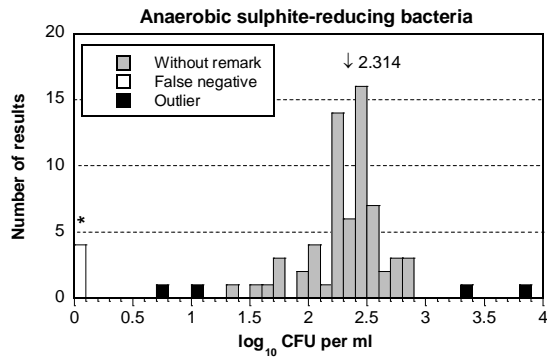
B



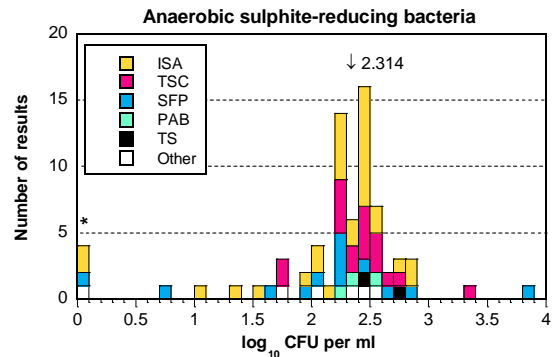
B



C



C



## **Aerobic microorganisms in fish products, 20-25 °C**

---

### **Mixture A**

The strains of *L. plantarum* and *E. coli* were present in the highest concentrations and thus most colonies were from these species. One low outlier was reported.

### **Mixture B**

The strains of *S. putrefaciens* and *S. aureus* were present in the highest concentrations and thus most colonies were from these species. During quality control at the National Food Agency the parameter did not fulfil the requirements for homogeneity, and a larger than usual distribution of the results could thus be expected. No values have therefore been considered as outliers. Laboratories with results lower than  $\log_{10} 3.0$  cfu ml<sup>-1</sup> should however still consider repeating the analysis. No false negative results were reported.

*Comment: All results in mixture B are considered correct. The results are not statistically evaluated. Therefore, no z-scores have been calculated for the results, and they are also not included in the tables located below the box plots.*

### **Mixture C**

The strains of *H. alvei* and *C. piscicola* were present in the highest concentrations and thus most colonies were from these species. Two low and one high outlier was reported.

### **General remarks**

Most laboratories (86 %) followed the method for aerobic microorganisms and spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with iron agar (IA), which was also the medium most frequently used by the laboratories (86 %). Two laboratories followed ISO 4833-1:2013 ("Colony count at 30 °C by the pour plate technique") and thus incubated on PCA. Two laboratories followed NMKL 86 ("Aerobic microorganisms in food"). Though this method is adapted for use in all types of food, it also refers to NMKL 184:2006 when analysing fish and fish products. One laboratory followed NMKL 96:2003, which uses the same method for total aerobic count as NMKL 184:2006. However, NMKL 96:2003 has been replaced by NMKL 96:2009 ("Coliform bacteria, thermotolerant coliform bacteria and *E. coli*") which refers to NMKL 184:2006 for the analysis of total aerobic count in fish and seafood.

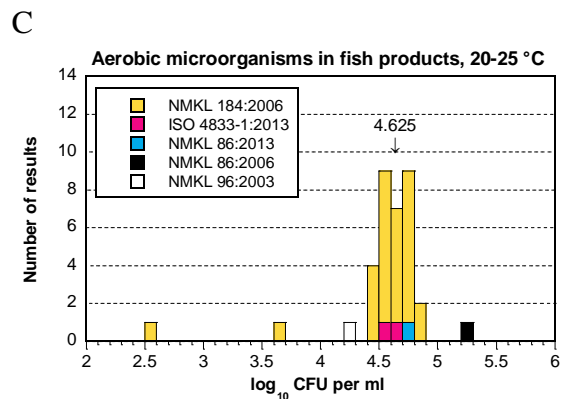
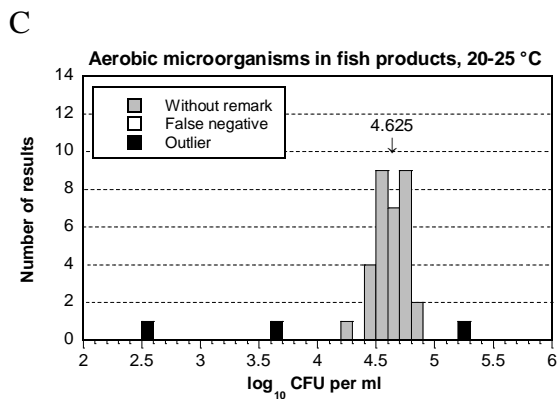
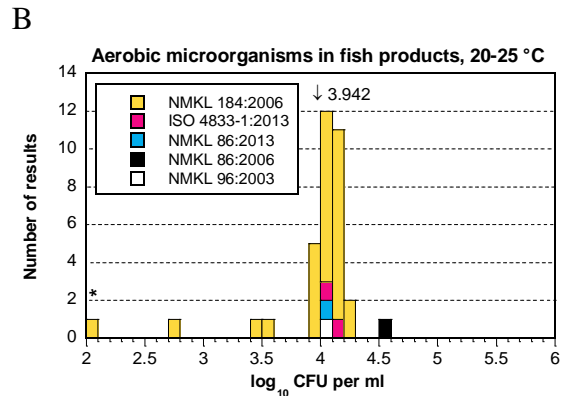
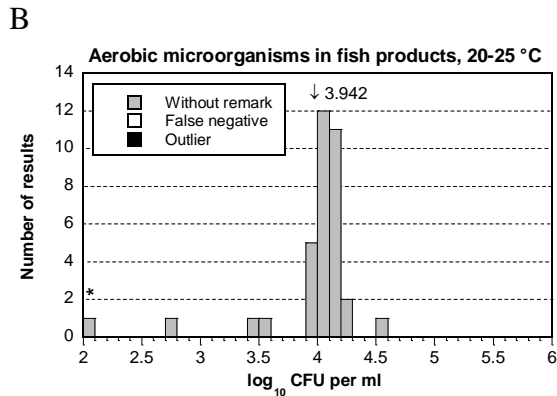
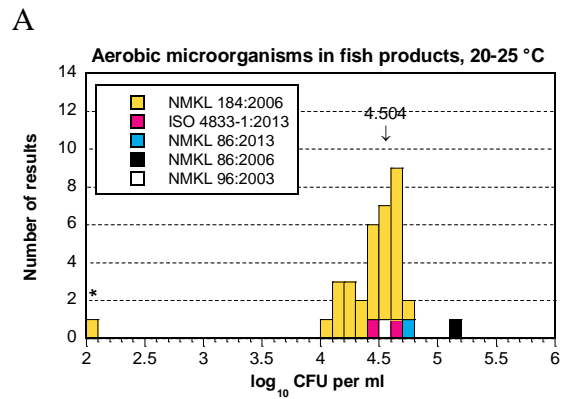
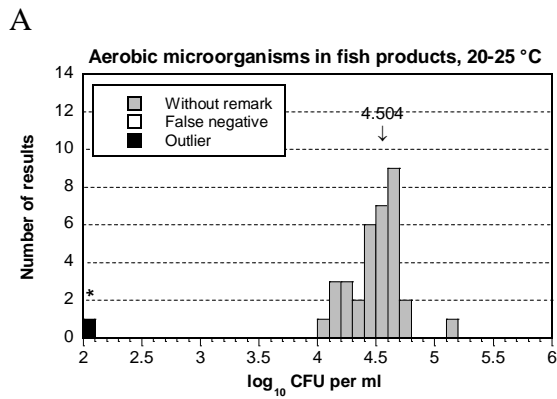
It could here be mentioned that NMKL 184:2006 also describes incubation on Long & Hammer agar for the detection of psychrotrophic and heat-sensitive microorganisms. With this medium, incubation is done at 15 °C, which may be advantageous when analysing fresh minced fish meat or lightly preserved fish products.

Since the majority of the laboratories followed NMKL 184:2006 and used IA, no differences between methods and media have been identified.

Results from analysis of aerobic microorganisms in fish products.

Method	N	Mixture A					Mixture B*					Mixture C							
		n	m	s	F	< >	n	m	s	F	< >	n	m	s	F	< >			
All results	35	34	4.504	0.215	0	1	0	35	3.942	0.510	0	0	0	32	4.625	0.131	0	2	1
NMKL 184:2006	30	29	4.472	0.190	0	1	0	30	3.907	0.539	0	0	0	28	4.635	0.117	0	2	0
ISO 4833-1:2013	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0
NMKL 86:2013	1	1	-	-	0	0	0	1	-	-	0	0	0	1	-	-	0	0	0
NMKL 86:2006	1	1	-	-	0	0	0	1	-	-	0	0	0	0	-	-	0	0	1
NMKL 96:2003	1	1	-	-	0	0	0	1	-	-	0	0	0	1	-	-	0	0	0

\* The results for mixture B are not evaluated.



## H<sub>2</sub>S-producing bacteria in fish products

### Mixture A

No target organism was present in the mixture. One laboratory reported a false positive result.

### Mixture B

The strain of *S. putrefaciens* was target organism. During quality control at the National Food Agency the parameter did not fulfil the requirements for homogeneity, and a larger than usual distribution of the results could thus be expected. No values have therefore been considered as outliers. No false negative results were reported.

*Comment: All results in mixture B are considered correct. The results are not statistically evaluated. Therefore, no z-scores have been calculated for the results, and they are also not included in the tables located below the box plots.*

### Mixture C

The strain of *H. alvei* was target organism. Four low outliers were reported.

### General remarks

The majority of the laboratories (94 %) followed the method for aerobic microorganisms and spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with iron agar (IA), which was also the medium most frequently used by the laboratories (94 %). One laboratory followed ISO 4833-1:2013 (“Colony count at 30 °C by the pour plate technique”) and thus incubated on PCA, which is not correct for this analysis. One laboratory followed NMKL 96:2003 (“Bacterial examinations in fresh and frozen seafood”), which includes analysis of H<sub>2</sub>S-producing bacteria. However the laboratory incubated in lauryl sulphate broth, which is incorrect. Further, NMKL 96:2003 has been replaced by NMKL 96:2009 (“Coliform bacteria, thermotolerant coliform bacteria and *E. coli*”) which refers to NMKL 184:2006 for the analysis of aerobic count and spoilage bacteria in fish and seafood.

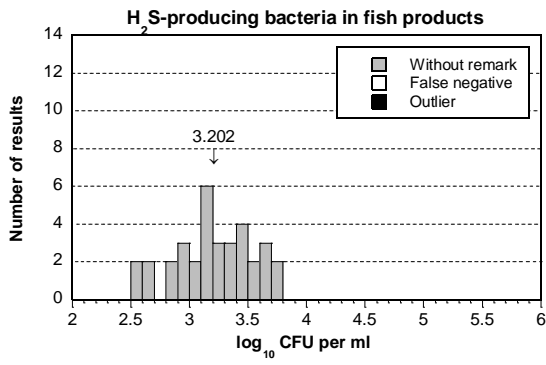
Since the majority of the laboratories followed NMKL 184:2006 and used IA, no differences between methods and media have been identified.

### Results from analysis of H<sub>2</sub>S-producing bacteria in fish products.

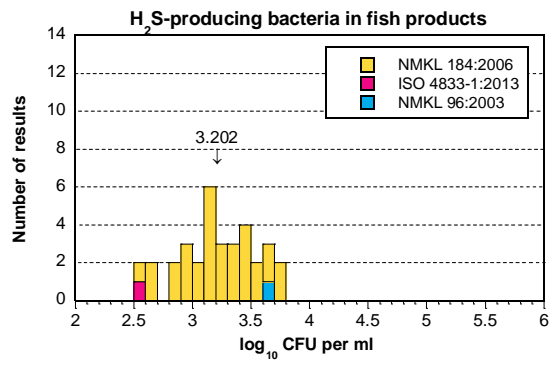
Method	N	Mixture A						Mixture B*						Mixture C					
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	34	33	-	-	1	-	-	34	3.202	0.337	0	0	0	30	4.284	0.104	0	4	0
NMKL 184:2006	32	31	-	-	1	-	-	32	3.210	0.317	0	0	0	29	4.289	0.102	0	3	0
ISO 4833-1:2013	1	1	-	-	0	-	-	1	-	-	0	0	0	0	-	-	0	1	0
NMKL 96:2003	1	1	-	-	0	-	-	1	-	-	0	0	0	1	-	-	0	0	0

\* The results for mixture B are not evaluated.

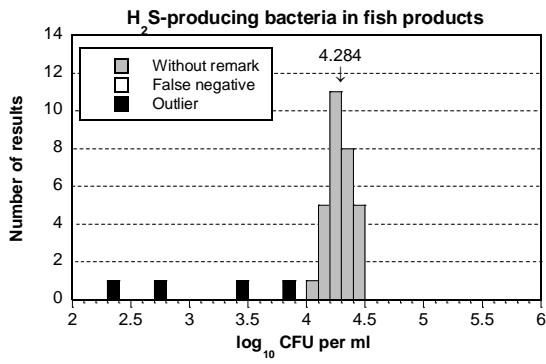
B



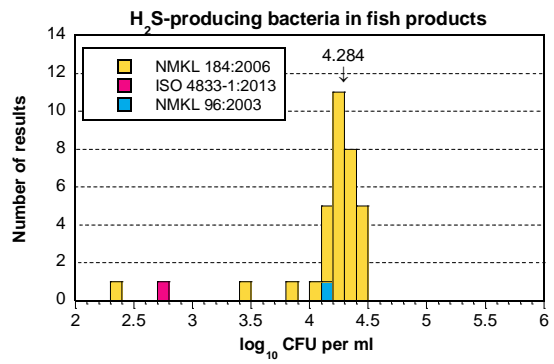
B



C



C



## Yeasts and moulds

---

### Mixture A

The strain of *K. marxianus* was target organism for the analysis of yeasts. Five high outliers were reported, as well as 18 false negative results. The strain of *K. marxianus* has in previous proficiency testing rounds been observed to form very small colonies on dichloran Rose-Bengal chloramphenicol agar (DRBC) and on dichloran glycerol agar (DG18). Laboratories that incubated on DG18 reported lower results compared to laboratories that used other media, and they also reported more false negative results. The false results associated with DG18 could however not be attributed to the use of a specific method. A disproportionately high number of false negative results were also reported by laboratories that used Petrifilm RYM and Petrifilm YM. This has not previously been observed for the strain of *K. marxianus* when using Petrifilm, and could thus possibly be simply due to chance.

The strain of *P. verrucosum* was target organism for the analysis of moulds. Seven low and three high outliers were reported, as well as nine false negative results. None of these could be associated with the use of a specific method or medium.

### Mixture B

The strain of *H. uvarum* was target organism for the analysis of yeasts. One high and five low outliers were reported, as well as two false negative results. In contrast to mixture A, the results for yeasts were more similar between the different media.

The mixture did not contain a target organism for the analysis of moulds. Six false positive results were reported, all of which were from different media.

### Mixture C

The mixture did not contain a target organism for the analysis of yeasts. Five false positive results were reported, all of which were from different media. In addition, one of the false results was from a laboratory that reported a combined value for yeasts and moulds.

A strain of *P. verrucosum* (not identical to that in mixture A) was target organism for the analysis of moulds. Nine low and two high outliers were reported, as well as ten false negative results. Here as well the outliers and false results were fairly evenly distributed among the various methods and media.

### General remarks

In principle the same laboratories analysed both yeast and moulds, and with identical methods in both analyses. The methods consisted of NMKL 98:2005, ISO 6611:2004 / IDF 94:2004, 3M™ Petrifilm™ and ISO 21527-1:2008 / ISO 21527-2:2008. Four laboratories followed ISO 7954:1987 ("General guidance for enumeration of yeasts and moulds"), which has been replaced by ISO 21527-1:2008 and ISO 21527-2:2008.

NMKL 98:2005 prescribes the use of either DRBC, DG18 or oxytetracyclin glucose yeast extract agar (OGYE). ISO 6611:2004/IDF 94:2004 describes the enumeration of yeasts and moulds in milk and milk products, and is based on a pour-plate method with OGYE or yeast extract glucose chloramphenicol agar (YGC). With ISO 21527 different media are used depending on the water activity ( $a_w$ ) of the food that is analysed, and ISO 21527-1:2008 therefore uses DRBC while ISO 21527-2:2008 uses DG18. In general, DRBC is recommended for fresh foods with  $a_w > 0,95$  (e.g. fruit, vegetables,

meat and milk products) and DG18 for foods with  $a_w \leq 0,95$  (e.g. dried fruits, dried meats, grains, nuts). OGYE is recommended if only yeasts are to be analysed.

Outliers and false results were more or less proportionally distributed between the different methods and media that were used. The mean values of the different groups were also generally similar. Further, many of the methods and media were in used only by a small number of laboratories. In addition to what has previously been mentioned it is therefore difficult to come to a conclusion regarding potential differences between the methods and media that were used.

Four and three laboratories used TEMPO<sup>®</sup> Yeast/Mold (TEMPO YM) for the analysis of yeasts and moulds, respectively. Three of these laboratories stated that they reported a combined value for yeasts and moulds, and these have hence often been outliers or false results for the respective analyses. The results from these three laboratories were excluded when identifying outliers, but they are otherwise shown in tables, figures and annex as they were reported by the laboratories. Reporting of a combined value for yeasts and moulds can currently not be handled in the statistical analysis – such results therefore need to be evaluated by the laboratories themselves.

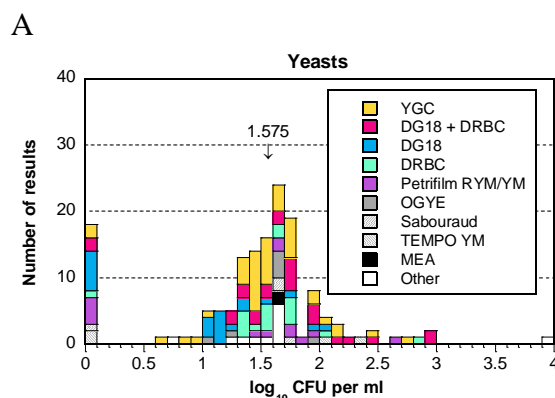
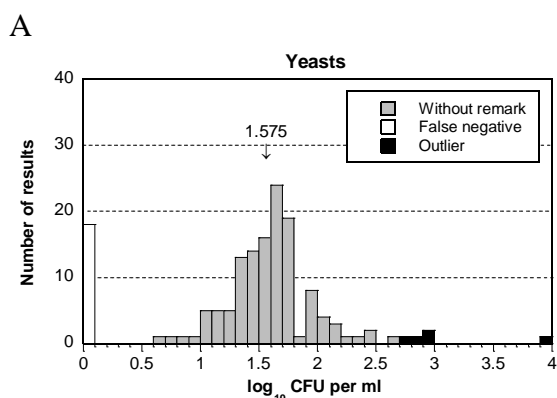
### Results from analysis of yeasts.

Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	149	126	1.575	0.332	18	0	5	144	3.340	0.202	2	5	1	145	-	-	5	-	-
YGC	43	40	1.556	0.353	2	0	1	41	3.289	0.198	0	2	0	41	-	-	1	-	-
DG18 + DRBC	25	21	1.690	0.314	2	0	2	24	3.325	0.191	1	1	0	25	-	-	1	-	-
DG18	21	15	1.316	0.321	6	0	0	19	3.348	0.191	0	1	1	21	-	-	0	-	-
DRBC*	18	16	1.570	0.191	1	0	1	19	3.478	0.206	0	0	0	17	-	-	1	-	-
Petrifilm RYM/YM	13	9	1.803	0.333	4	0	0	14	3.308	0.154	0	0	0	14	-	-	0	-	-
OGYE	7	7	1.566	0.310	0	0	0	7	3.341	0.214	0	0	0	7	-	-	0	-	-
Sabouraud**	4	3	-	-	1	0	0	4	-	-	0	0	0	4	-	-	0	-	-
TEMPO YM	4	2	-	-	2	0	0	4	-	-	0	0	0	3	-	-	1	-	-
MEA	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	-	-
Other***	12	11	1.461	0.279	0	0	1	10	3.326	0.233	1	1	0	11	-	-	1	-	-

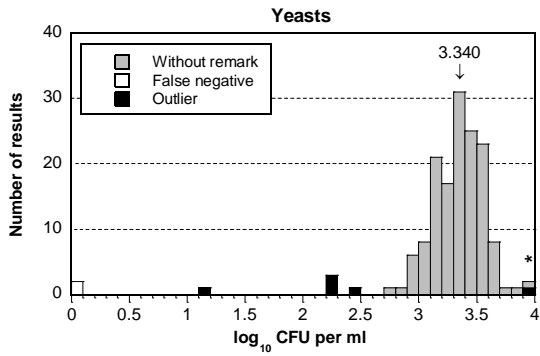
\* The group DRBC includes two laboratories that stated the use of Rose-Bengal chloramphenicol agar (RBC).

\*\* The group Sabouraud includes both Sabouraud chloramphenicol agar and Sabouraud dextrose agar.

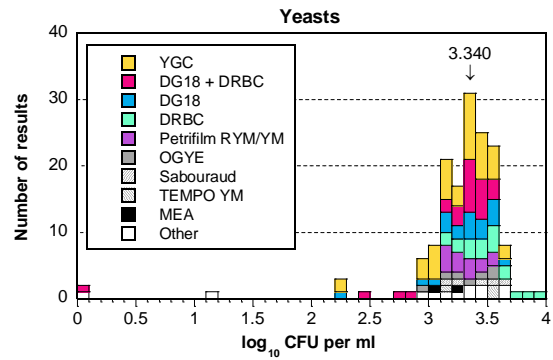
\*\*\* The group Other mainly includes laboratories that used a unique medium or medium combination.



B



B



*Results from analysis of moulds.*

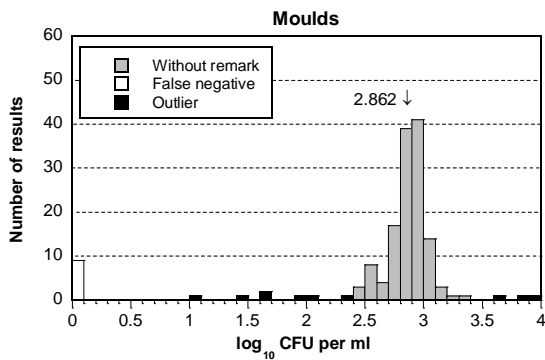
Medium	N	Mixture A					Mixture B					Mixture C							
		n	m	s	F	<	>	n	m	s	F	<	>	n	m	s	F	<	>
All results	150	131	2.862	0.153	9	7	3	143	-	-	6	-	-	131	2.739	0.130	10	9	2
YGC	46	41	2.793	0.171	4	1	0	43	-	-	1	-	-	40	2.737	0.119	3	3	0
DG18 + DRBC	26	22	2.885	0.111	1	2	1	26	-	-	1	-	-	25	2.741	0.131	1	1	0
DG18	21	18	2.847	0.155	0	1	2	21	-	-	0	-	-	18	2.706	0.142	0	1	2
DRBC*	18	18	2.943	0.098	0	0	0	16	-	-	1	-	-	17	2.819	0.118	1	0	0
Petrifilm RYM/YM	11	9	2.877	0.145	2	0	0	11	-	-	1	-	-	8	2.726	0.169	3	1	0
OGYE	6	6	2.902	0.071	0	0	0	6	-	-	0	-	-	6	2.768	0.053	0	0	0
TEMPO YM	3	1	-	-	1	1	0	2	-	-	1	-	-	0	-	-	1	2	0
Sabouraud**	3	3	-	-	0	0	0	3	-	-	0	-	-	3	-	-	0	0	0
PDA	3	2	-	-	0	1	0	3	-	-	0	-	-	3	-	-	0	0	0
Other***	13	11	2.895	0.088	1	1	0	12	-	-	1	-	-	11	2.674	0.144	1	1	0

\* The group DRBC includes two laboratories that stated the use of Rose-Bengal chloramphenicol agar (RBC).

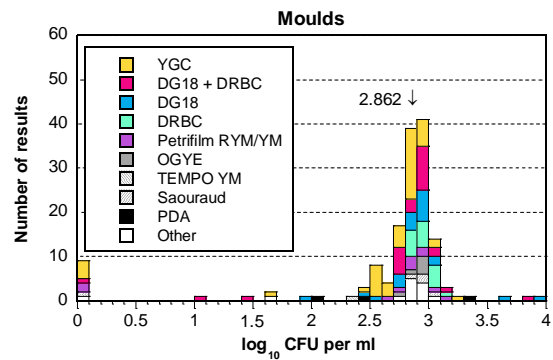
\*\* Sabouraud chloramphenicol agar.

\*\*\* The group Other mainly includes laboratories that used a unique medium or medium combination.

A

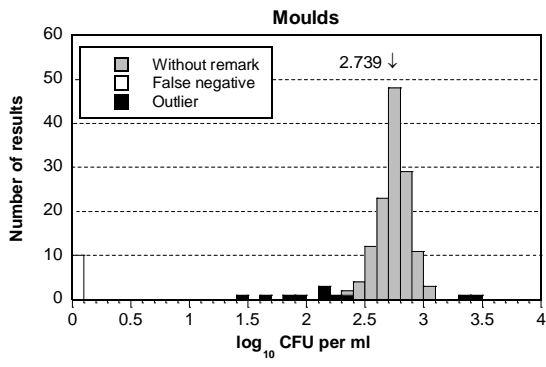


A

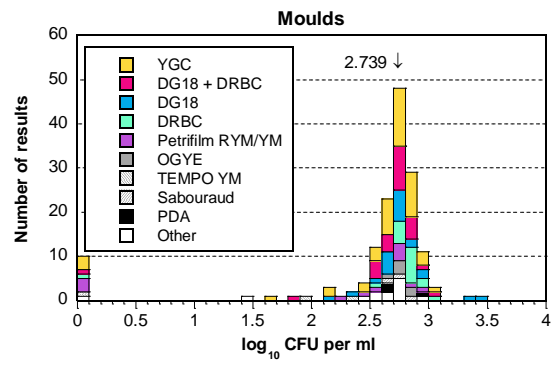




C



C



## **Outcome of the results of individual laboratory - assessment**

### **Reporting and evaluation of results**

The reported results of all participating laboratories are listed in Annex 1, together with the minimum and maximum accepted values for each analysis. Results that received a remark (false results and outliers) are highlighted in yellow, with bold font.

It is the responsibility of the participating laboratories to correctly report results according to the instructions. When laboratories incorrectly report their results, for example by stating “pos” or “neg” for quantitative analyses, the results cannot be correctly processed. Such incorrectly reported results are normally excluded. Inclusion and further processing of such results may still be done, after manual assessment in each individual case.

Z-scores (see below) for individual analyses are shown in Annex 2 and can be used as a tool by laboratories when following up on the results.

The laboratories are not grouped or ranked based on their results. The performance of a laboratory as a whole can be evaluated from the number of false results and outliers that are listed in Annex 1 and below the box plots.

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

### **Z-scores, box plots and deviating results**

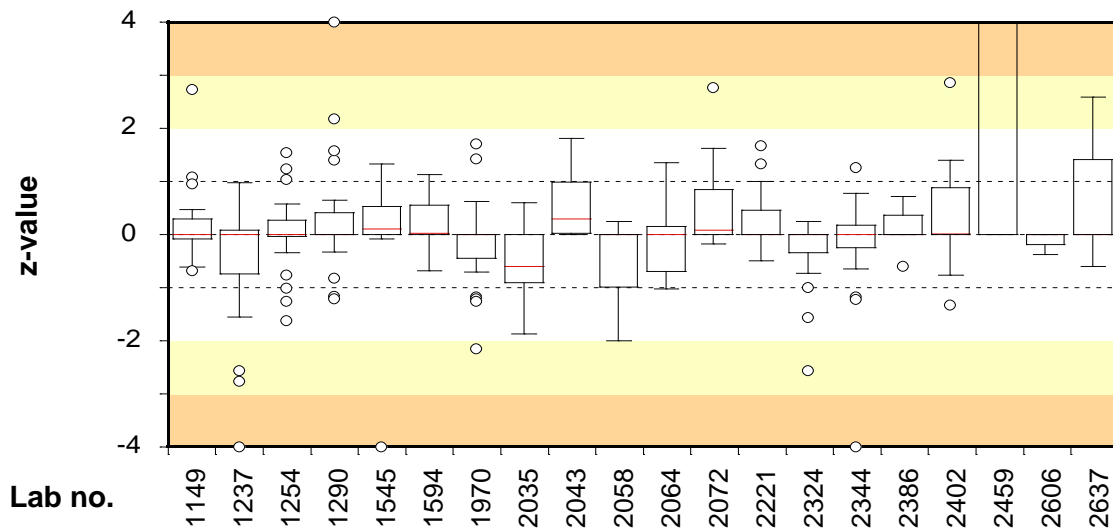
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated from all laboratory results for each analysis. False results do not generate any z-scores.

The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates the results of that individual laboratory, with false results excluded, are close to the general mean values calculated for all laboratory results. The range of z-scores is indicated by the size of the box and, for most laboratories, by lines and/or circles above and beneath the box. For each laboratory, the number of false results and outliers are also listed in the tables below the box plots.

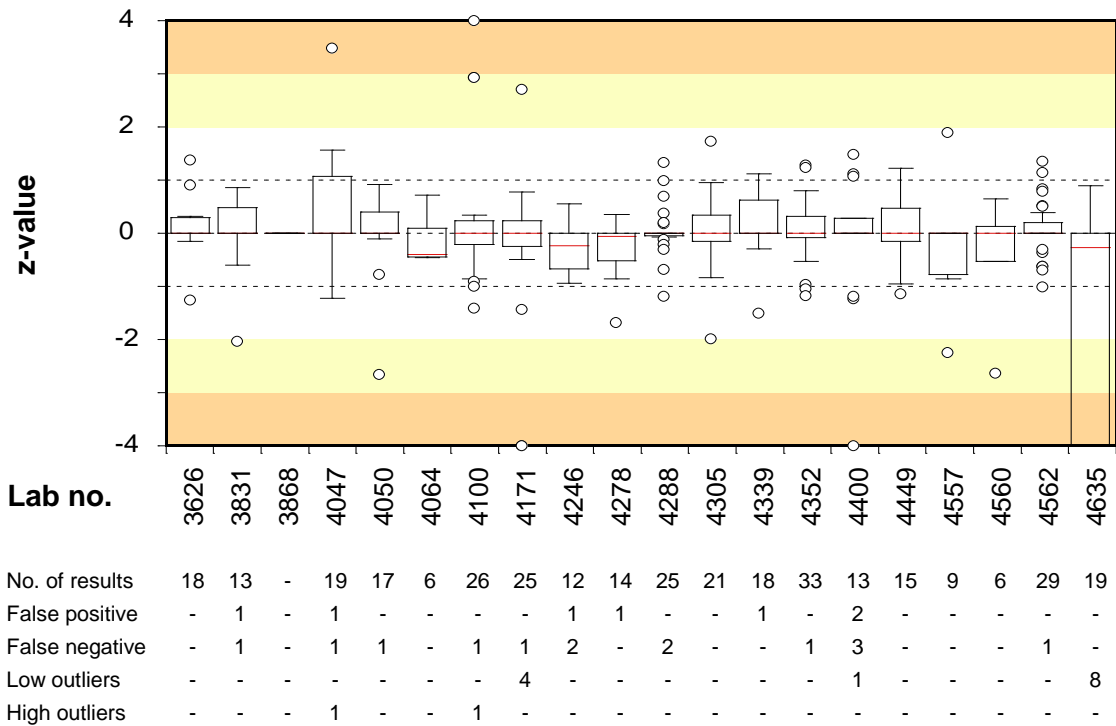
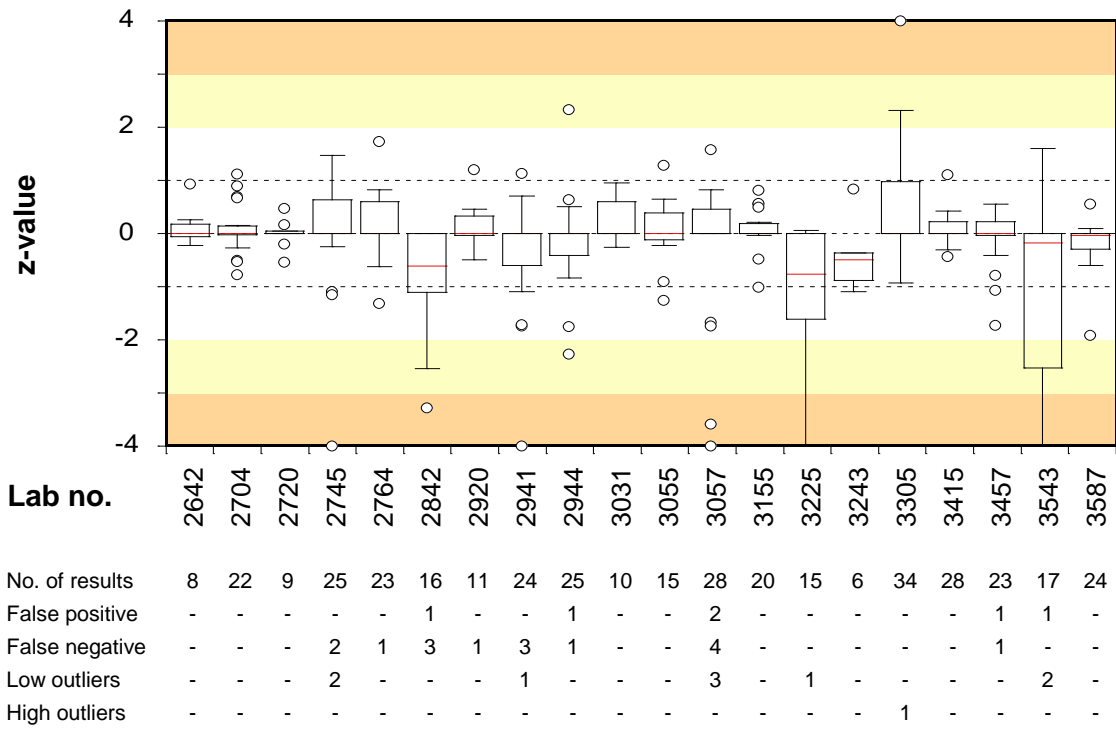
### Box plots and numbers of deviating results for each laboratory

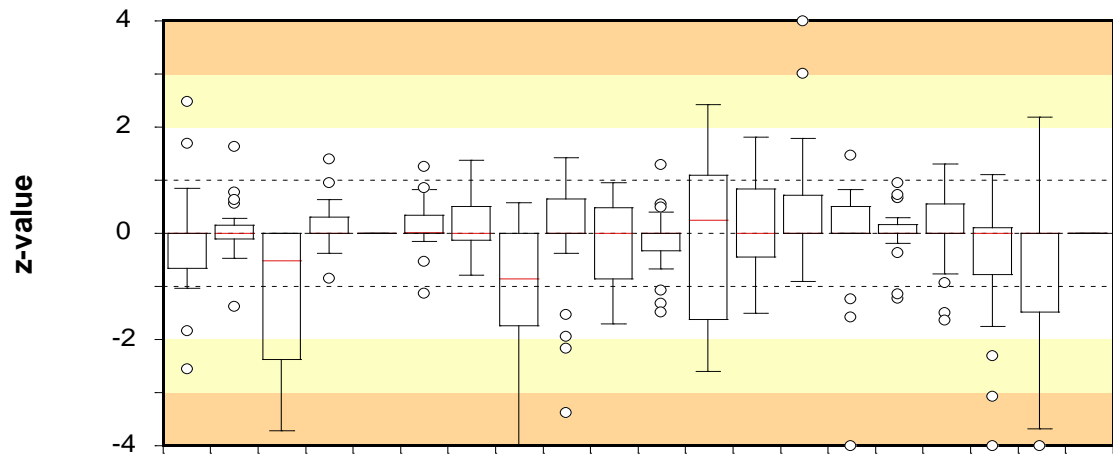
- Z-scores are calculated according to the formula:  $z = (x-m)/s$ , where  $x$  is the result of the individual laboratory,  $m$  is the mean of the results of all participating laboratories, and  $s$  is the standard deviation of the participating laboratories, after removing outliers and false results.
- Outliers are included in the figures after being calculated to z-scores in the same way as for other results.
- False results do not generate any z-scores, and are not included in “No. of results”.
- Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism generate a z-score of 0.
- The laboratory median value is illustrated by a horizontal red line in the box.
- The box includes 50 % of a laboratory’s results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box.
- A circle is for technical reasons shown in the plot when a value deviates to certain degree\* from the other values. This does not by itself indicate that the value is an outlier.
- z-scores  $>+4$  and  $<-4$  are positioned at  $+4$  and  $-4$ , respectively, in the plot.
- The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

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

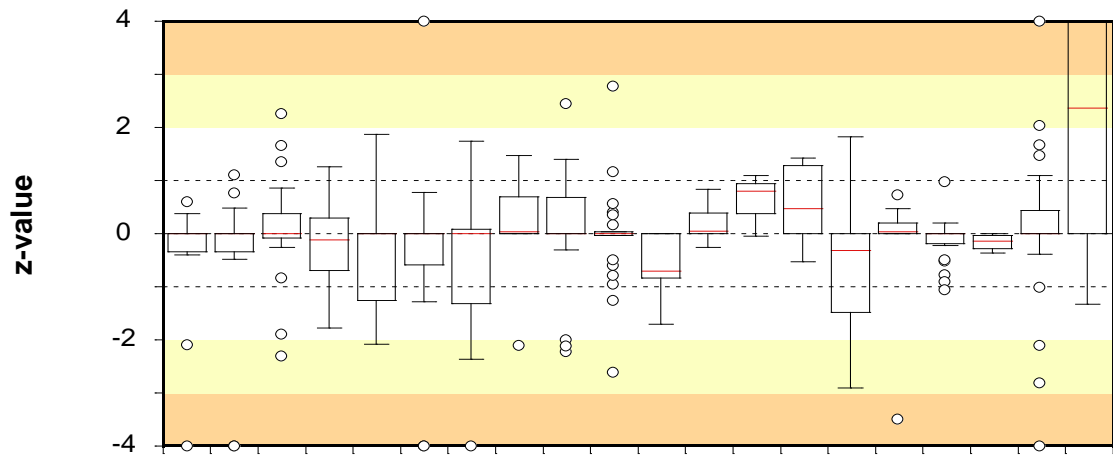


Lab no.	1149	1237	1254	1290	1545	1594	1970	2035	2043	2058	2064	2072	2221	2324	2344	2386	2402	2459	2606	2637
No. of results	18	32	27	23	28	26	36	6	8	13	15	30	28	22	24	9	14	20	3	28
False positive	-	2	-	-	1	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-
False negative	-	2	1	1	1	-	-	-	1	2	-	1	1	-	-	-	1	1	-	2
Low outliers	-	3	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
High outliers	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	-

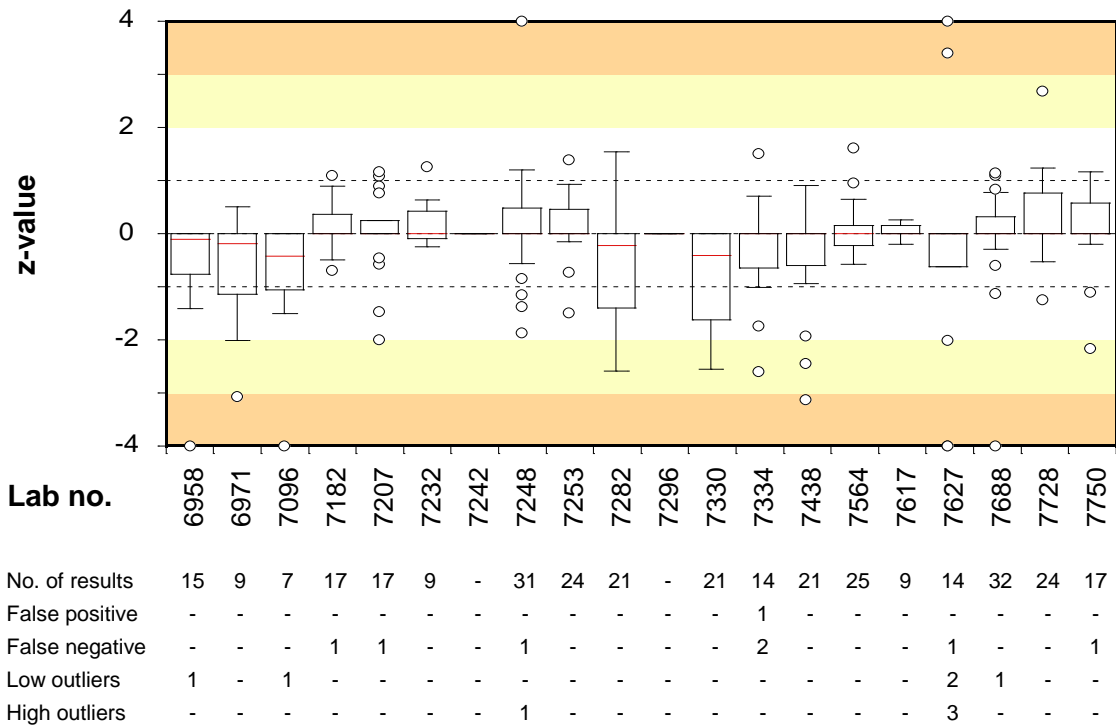
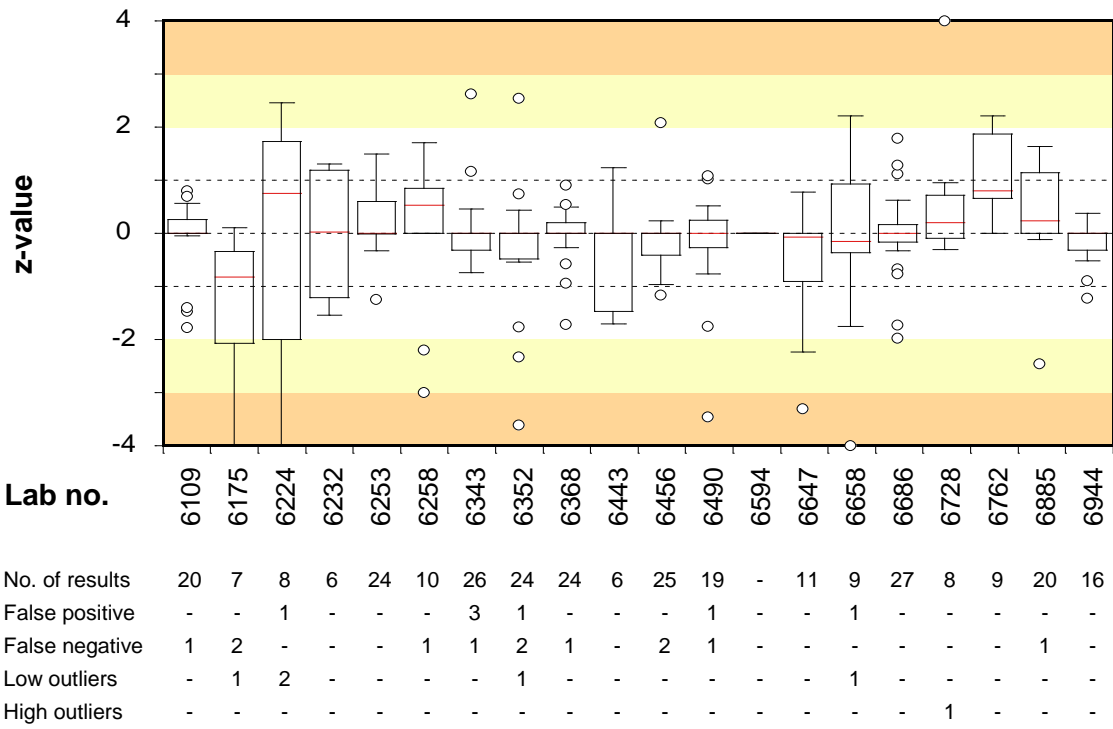


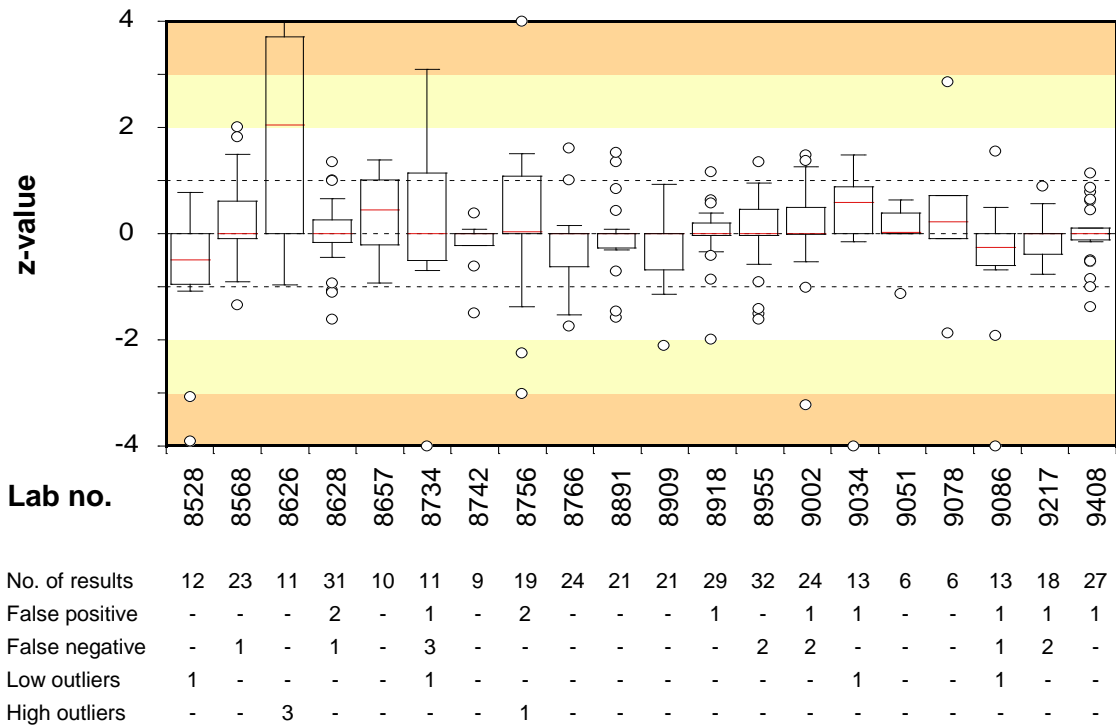
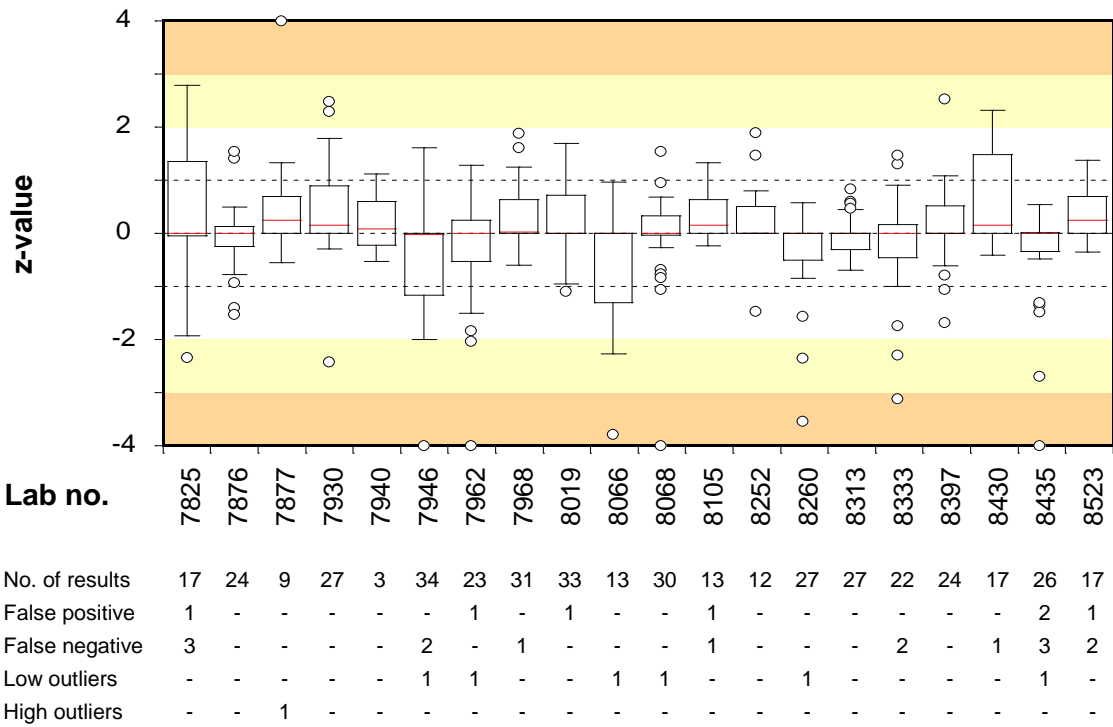


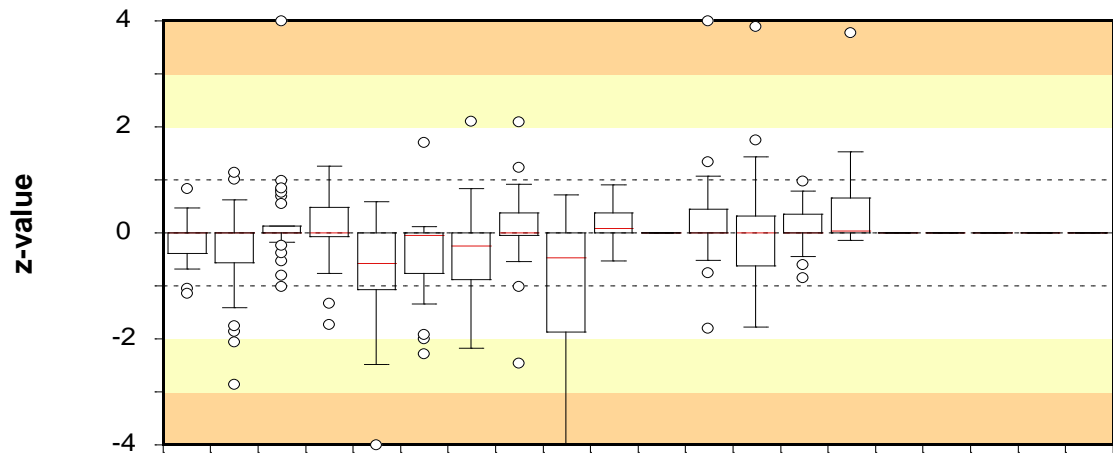
Lab no.	4664	4817	4840	4878	4879	4889	4944	4951	4980	4998	5018	5100	5119	5120	5182	5188	5201	5204	5220	5221
No. of results	24	20	4	15	-	22	28	15	29	4	29	6	11	34	14	30	21	31	21	-
False positive	-	1	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-	1	-	-
False negative	1	-	1	-	-	-	-	-	1	5	1	-	3	-	1	4	-	-	-	-
Low outliers	-	-	1	-	-	-	-	2	1	-	-	-	-	-	1	-	-	3	4	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-



Lab no.	5250	5329	5333	5338	5342	5352	5494	5545	5553	5615	5632	5654	5701	5774	5801	5808	5883	5933	5950	5993
No. of results	16	20	24	12	18	23	15	14	18	26	5	14	3	6	14	10	24	4	36	16
False positive	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
False negative	2	3	-	-	-	1	-	1	-	1	-	1	-	-	1	2	-	-	-	-
Low outliers	2	2	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	2	-
High outliers	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	7







**Lab no.**

9429 9436 9441 9453 9512 9555 9559 9662 9747 9783 9853 9886 9890 9903 9950

No. of results	21	32	30	18	14	21	26	30	14	9	-	29	22	24	12
False positive	-	-	-	-	1	-	-	-	-	-	-	1	1	-	2
False negative	-	-	-	-	-	-	1	-	1	-	-	2	1	-	1
Low outliers	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-
High outliers	-	-	1	-	-	-	-	-	-	-	-	1	1	-	1



## Test material and quality control

### Test material

Each laboratory received three manufactured freeze-dried microbial mixtures, designated A-C. The test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (8). Before analysing the samples, the contents of each vial had to be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

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

Mixture <sup>1</sup>	Microorganism	Strain	
		SLV no. <sup>2</sup>	Reference <sup>3</sup>
A	<i>Escherichia coli</i>	SLV-524	CCUG 47554
	<i>Kluyveromyces marxianus</i>	SLV-439	CBS G99-106
	<i>Lactobacillus plantarum</i>	SLV-445	ATCC 8014
	<i>Penicillium verrucosum</i>	SLV-526	CBS 111026
B	<i>Clostridium perfringens</i>	SLV-442	CCUG 43593
	<i>Hanseniaspora uvarum</i>	SLV-555	-
	<i>Serratia marcescens</i>	SLV-040	ATCC 13880
	<i>Shewanella putrefaciens</i>	SLV-520	CCUG 46538
	<i>Staphylococcus aureus</i>	SLV-539	-
	<i>Bacillus cereus</i>	SLV-517	CCUG 44739
C	<i>Carnobacterium piscicola</i>	SLV-519	CCUG 46539
	<i>Clostridium bifermentans</i>	SLV-009	CCUG 43592
	<i>Hafnia alvei</i>	SLV-015	CCUG 45642
	<i>Penicillium verrucosum</i>	SLV-544	CBS 112488

<sup>1</sup> The links between the mixtures and the randomised sample numbers are shown in Annex 1.

<sup>2</sup> Internal strain identification no. at the National Food Agency

<sup>3</sup> Origin or culture collection (CCUG: Culture Collection University of Gothenburg, Sweden ; ATCC: American Type Culture Collection, CBS: Westerdijk Fungal Biodiversity Institute)

### Quality control of the mixtures

It is essential to have aliquots of homogeneous mixture and equal volume in all vials in order to allow comparison of all freeze-dried samples from one mixture. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the mixtures or on 5 vials if an “old” mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a mixture is approved if, for each analysis, the values obtained for the test of reproducibility (T) and the test “Index of dispersion” between vials (I<sub>2</sub>) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and I<sub>2</sub>, see references 9 and 10 respectively.)

**Table 3.** Concentration mean (m), T and I<sub>2</sub> values from the quality control of the mixtures; m is expressed in log<sub>10</sub> cfu (colony forming units) per ml of sample.

Analysis and method	A <sup>1</sup>			B <sup>2</sup>			C <sup>1</sup>		
	m	T	I <sub>2</sub>	m	T	I <sub>2</sub>	m	T	I <sub>2</sub>
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.708	1.46	1.699	5.162	1.36	3.577	4.749	1.19	0.408
Psychrotrophic microorganisms NMKL method no. 86:2013	2.846	1.23	0.761	4.478	1.87	2.994	4.772	1.34	1.239
Enterobacteriaceae NMKL method no. 144:2005	4.249	1.26	1.080	2.960	1.10	0.219	4.338	1.14	0.450
<i>Escherichia coli</i> NMKL method no. 125:2005	4.265	1.37	2.430	-	-	-	-	-	-
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	-	-	-	-	-	-	3.512	1.38	0.882
Coagulase-positive staphylococci NMKL method no. 66:2009	-	-	-	4.102	1.63	0.809	-	-	-
Lactic acid bacteria NMKL method no. 140:2007	4.481	1.23	0.321	-	-	-	4.228	1.54	0.783
<i>Clostridium perfringens</i> NMKL method no. 95:2009	-	-	-	2.674	1.19	0.381	-	-	-
Anaerobic sulphite-reducing bacteria NMKL method no. 56:2015	-	-	-	2.836	1.33	1.552	2.394	1.50	1.112
Aerobic microorganisms in fish products NMKL method no. 184:2006	4.682	1.31	0.837	4.044 <sup>3</sup>	3.52 <sup>3</sup>	33.39 <sup>3</sup>	4.693	1.17	0.306
H <sub>2</sub> S-producing bacteria in fish products NMKL method no 184:2006	-	-	-	3.885 <sup>3</sup>	3.31 <sup>3</sup>	23.80 <sup>3</sup>	4.381	1.16	0.133
Yeasts NMKL method no. 98:2005, DRBC	1.991	1.75	0.679	3.446	1.55	1.368	-	-	-
Moulds NMKL method no. 98:2005, DRBC	2.995	1.47	3.877	-	-	-	2.913	1.58	1.957

– No target organism and therefore no value

<sup>1</sup> n = 5 vials analysed in duplicate

<sup>2</sup> n = 10 vials analysed in duplicate

<sup>3</sup> Does not fulfil the homogeneity criteria; the parameter is not evaluated.

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Lab no.	Vial	Aerobic microorganisms 30 °C			Psychrotroph. microorganisms			Enterobacteriaceae			<i>Escherichia coli</i>			Presumptive <i>Bacillus cereus</i>			Coagulase-positive Staphylococci			Lactic acid bacteria			<i>Clostridium perfringens</i>			Anaerobic sulphite-red. bacteria			Aerobic m.o. in fish products 20-25 °C			H <sub>2</sub> 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				
6224	1 3 2	1.713	-4.000	2.456				-4.000	1.495	1.758			0	0.023																						6224					
6232	1 2 3	0.999	-1.215	-0.952				-1.545	1.310	1.186																										6232					
6253	2 1 3	-0.305	-1.247	0.878				0.300	1.495	0.159	0.672	0	0	0	-0.065	0	-0.325	0				0	-0.033	0.566											6253						
6258	1 3 2	1.704	0.390	1.234																																6258					
6343	1 3 2	1.170	-0.362	-0.147				-0.028	-0.424	0.159	-0.026	0	0	0	0.466	0	2.632	0	-0.148		0	0.337		0	-0.317	-0.433									6343						
6352	1 3 2	-0.538	0.442	0.089				0.431		-0.334	-0.491	0	0	0	-2.323	0	-1.759	0	-0.461	0		0	2.545													6352					
6368	2 3 1	0.005	0.201	-0.936				0.169	0.416	0.159	0.497	0	0			0	0.213	0	-0.273	0							-0.576	-1.717	0	-0.235			0.903	0	0.545	6368					
6443	2 1 3																																				6443				
6456	3 2 1	0.238	-1.167	-0.936				-0.357	-0.963	-0.764	-0.200	0	0	0	0		0	-0.415	0		0	2.086	0	0	-0.887	-0.272										6456					
6490	1 3 2	0.316	0.523	-0.147				1.022	0.176	-0.764																											6490				
6594	3 1 2																																				6594				
6647	2 3 1	0.782	-0.684	-0.069																																	6647				
6658	1 2 3	-0.150	0.925	0.089				-0.357	-0.304	-4.000																											6658				
6686	1 2 3	0.394	-0.041	1.115	1.279	0.045		-0.291	-0.304	-0.026	0.032	0	0																								6686				
6728	2 1 3	-0.150	4.000	0.089				0.957	-0.304	-0.026	0.323																										6728				
6762	1 3 2	1.868	2.212	0.799				1.548	0.656	0.712	2.124	0	0																								6762				
6885	3 2 1	1.636	0.684	0.089				1.088	1.555	0.589																												6885			
6944	1 2 3										0.265	0	0																								6944				
6958	3 2 1	-0.150	-4.000	-1.410				-0.751	-0.004	-0.764																												6958			
6971	3 1 2	-2.013	0.362	-3.066				-1.145	-0.184	-0.457																												6971			
7096	2 1 3										-0.607	0	0																									7096			
7182	2 3 1	-0.693	0.362	0.010				-0.488	0.896	0.343	-0.316	0	0																									7182			
7207	1 2 3	-1.469	1.086	0.247				-1.998	0.896	-0.580																												7207			
7232	3 2 1	-0.096	-0.177	-0.242																																		7232			
7242	2 1 3																																					7242			
7248	2 3 1	0.238	0.281	0.247				0.497	-0.844	-1.872	0.497	0	0	0	0.466	0	1.198	0	0.917	0	0.902																7248				
7253	2 1 3	0.316	0.442	0.484				0.300	0.596	-0.149	-0.723	0	0																									7253			
7282	1 3 2	-1.858	-1.247	-2.514				-0.225	-0.963	-1.072	-2.583	0	0	0	-1.393	0	-2.027	0																			7282				
7296	2 3 1																																					7296			
7330	1 2 3	-1.625	-1.408	-0.384				-2.523	-1.983	-0.272	-2.525	0	0	0	-1.393	0	-1.311	0																			7330				
7334	2 3 1	0.685	-0.650	0.706																																		7334			
7438	1 2 3	0.161	-0.604	-0.226				-0.028	-1.923	-0.211	-0.200	0	0																									7438			
7564	1 2 3	-0.227	-0.362	0.957																																			7564		
7617	2 3 1	0.005	-0.202	0.168							0.149	0	0																									7617			
7627	1 3 2	-2.013	-4.000	-0.621																																		7627			
7688	2 1 3	1.092	0.201	0.484	-0.226	0.610		-0.291	0.116	-0.149	0.323	0	0	0	-1.128	0	0.840	0	-0.085	0	1.138																7688				
7728	3 1 2	1.170	-1.247	1.036				1.020	0	0	0	2.679	0	0			0.213	0																				7728			
7750	3 2 1	-2.168	-0.202	0.247																																			7750		
7825	1 2 3	-0.041	2.783	1.351				0.891	1.567	1.463																													7825		
7876	3 2 1	-1.392	0.281	-0.305				1.416	-0.604	0.220	0.032	0	0	0	-1.526	0	-0.773	0																				7876			
7877	1 3 2	1.325	4.000	0.247				0.694	0.236	0.466																													7877		
7930	1 3 2	2.489	0.120	0.720				0.694	1.076	0.343	2.299	0	0	1.794																								7930			
7940	1 3 2	0.083	-0.523	1.115																																			7940		
7946	3 1 2	-1.625	-1.167	-1.410				-1.998	1.615	-0.518	-1.711	0	0	0	-0.729	0	-0.056	0	-0.587	0																		7946			
7962	2 1 3	0.471	0.281	0.563				-0.357	-1.503	0.712	0.207	0	0	0	-4.000	0	0.482	0	-2.028		-0.646																	7962			
7968	1 2 3	0.394	-0.604	0.720				1.613	0.116	0.835	1.253	0	0	0	0.023	0	-0.146	0	0.228	0																		7968			
8019	1 2 3	0.782	0.040	0.720				-0.160	-0.244	0.835	0.904	0	0	0	0.953	0	0.930	0	-1.088	0	-0.310	0	0.108														8019				
8066	2 3 1										0.962	0	0																										8066		
8068	2 1 3	0.316	-0.684	0.089				-0.028	0.356	0.959	1.543	0	0	0	0.687	0	-4.000	0	-0.273	0	0.633																	8068			
8105	1 3 2	1.015	1.327	1.273							0.381	0	0																										8105		
8252	2 1 3	-1.469	1.890	0.799							0.207	0	0	0	0.200	0	1.467	0																				8252			
8260	3 1 2	-0.771	-0.845	-2.356				-0.816	-3.542	-1.564	0.381	0	0	0	0.244	0	0.571	0																				8260			
8313	3 2 1	0.238	-0.604	-0.384				-0.291	0.836	-0.395	-0.316	0	0	0	0.599	0	0.571	0																				8313			
8333	2 3 1	-1.004	-0.845	0.168				-1.736	-3.122	-0.457	-2.292	0	0	0	0.244																							8333			
8397	2 1 3	0.626																																							



## **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.

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### **The National Food Agency's reference material**

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

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