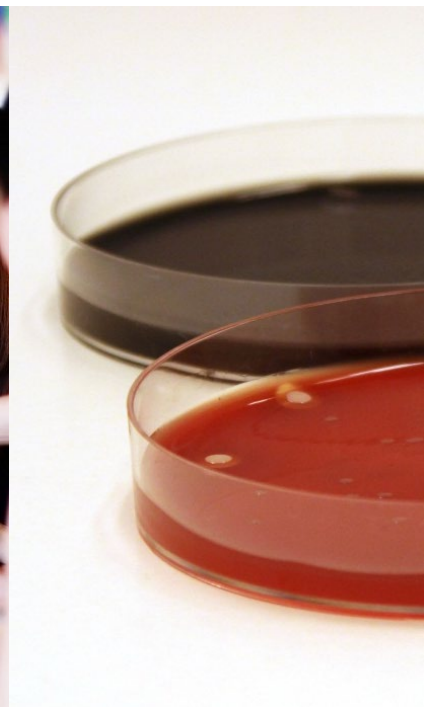


## Food Microbiology

April 2020

Jonas Ilbäck



*Edition*

Version 1 (2020-06-15)

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PT April 2020 is registered as no. 2020/00833 at the Swedish Food Agency

*Proficiency Testing*  
**Microbiology – Food**  
April 2020

**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

### Media

BA	Blood agar
BcsA	<i>Bacillus cereus</i> selective agar
BP	Baird-Parker agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
DG18	Dikloran 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
LSB	Lauryl sulphate broth
LTLSB	Lactose tryptone lauryl sulphate broth
mCP	Membrane Clostridium perfringens 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
PDA	Potato dextrose agar
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar
Petrifilm AC	3M™ Petrifilm™ Aerobic Count
Petrifilm Disk	3M™ Petrifilm™ Staph Express Disk
Petrifilm EB	3M™ Petrifilm™ Enterobacteriaceae
Petrifilm EC/CC	3M™ Petrifilm™ <i>E. coli</i> /Coliform Count
Petrifilm LAB	3M™ Petrifilm™ Lactic Acid Bacteria
Petrifilm SEC	3M™ Petrifilm™ Select <i>E. coli</i>
Petrifilm Staph	3M™ Petrifilm™ Staph Express
PCA	Plate count agar
RPFA	Baird-Parker agar with rabbit plasma fibrinogen
Saubouraud	Saubouraud chloramphenicol agar
SC	Sulphite cycloserine agar
SFP	Shahidi-Ferguson Perfringens agar
SPS	Sulphite polymyxin sulfadiazine agar
TBX	Tryptone bile X-glucuronide agar
TEMPO AC	TEMPO® Aerobic Count
TEMPO BC	TEMPO® <i>Bacillus cereus</i>
TEMPO EB	TEMPO® Enterobacteriaceae
TEMPO YM	TEMPO® Yeast/Mold
TGE	Tryptone glucose extract agar
TS	Tryptose sulphite agar
TSA	Trypton 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/Swedish Food Agency, Sweden

## Contents

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General information on results evaluation.....	6
Results of the PT round April 2020.....	7
- General outcome .....	7
- Aerobic microorganisms, 30 °C.....	8
- Psychrotrophic microorganisms .....	10
- Enterobacteriaceae .....	12
- <i>Escherichia coli</i> .....	13
- Presumptive <i>Bacillus cereus</i> .....	15
- Coagulase-positive staphylococci.....	18
- Lactic acid bacteria .....	20
- <i>Clostridium perfringens</i> .....	22
- Anaerobic sulphite-reducing bacteria .....	24
- Aerobic microorganisms in fish products, 20-25 °C .....	26
- H <sub>2</sub> S-producing bacteria in fish products .....	27
- Yeasts and moulds .....	29
Outcome of the results of individual laboratory – assessment .....	33
- Box plot .....	34
Test material and quality control .....	40
- Test material .....	40
- Quality control of the mixtures .....	41
References .....	42
Annex 1: Results obtained by the participants	
Annex 2: z-scores of all participants	

## General information on results evaluation

### Statistical evaluation of the results

For analyses, where more than 20 laboratories have reported results, outliers are identified with statistical methods. Values that after  $\log_{10}$  transformation do not belong to a strictly normal distribution are for this purpose identified as outliers with Grubbs' test modified by Kelly (1). When fewer than 20 laboratories have reported results, as well as in some individual cases, subjective adjustments are instead made to set outlier limits based on knowledge of the samples contents.

Mean values and standard deviations are normally provided for the different analyses. For analyses with fewer than 20 reported results, the median is provided instead of the mean value. Normally, for method groups with fewer than 5 results, only the number of false results and outliers are provided. Outliers and false results are not included in the calculations of mean values and standard deviations. Results reported as "> value" are not evaluated. Results reported as "< value" are interpreted as zero (negative result).



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. This method information is sometimes difficult to interpret, for example when laboratories state a medium that is not included in the standard method they refer to. In such cases, as a general rule, the medium stated by the laboratory is nevertheless used in method comparisons. Method data from laboratories that are in other ways contradictory or difficult to interpret are normally either excluded or added to the group "Other", together with results from methods and media that are only used by 1-2 laboratories.

### Uncertainty of measurement for the assigned values

The measurement uncertainty 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 is the mean value of the participants' results with outliers and false results excluded.




### 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 <sup>-1</sup> (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

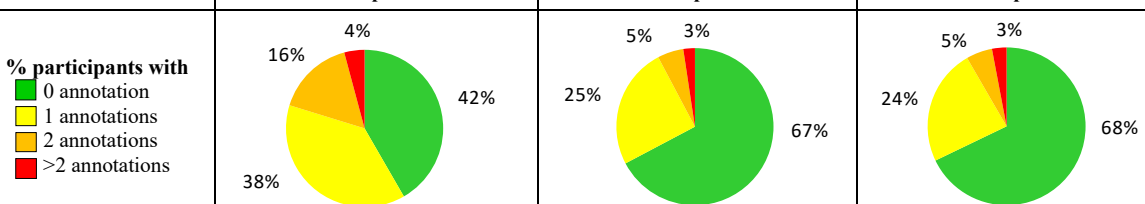
# Results of the PT round April 2020

## General outcome

Samples were sent to 184 laboratories, 40 in Sweden, 128 in other European countries, and 16 outside of Europe. Of the 168 laboratories that reported results, 126 (75 %) provided at least one result that received an annotation. In the previous round with similar analyses (April 2019) the proportion was 51 %. In total, somewhat fewer laboratories than usual reported results. As communicated by several participants, this is a consequence of limited laboratory capacity during the Covid-19 pandemic.

Individual results for each analysis in the PT round are listed in Annex 1 and are also available on the website after logging in: <https://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)

	Sample A				Sample B				Sample C			
	Target	N	F	X	Target	N	F	X	Target	N	F	X
<b>% participants with</b>												
<b>Microorganisms</b>	<i>Aeromonas hydrophila</i> <i>Clostridium perfringens</i> <i>Staphylococcus warneri</i> <i>Staphylococcus aureus</i> <i>Shewanella putrefaciens</i>				<i>Aspergillus flavus</i> <i>Bacillus cereus</i> <i>Brochothrix thermosphacta</i> <i>Clostridium perfringens</i> <i>Hanseniaspora uvarum</i> <i>Shewanella putrefaciens</i>				<i>Bacillus cereus</i> <i>Cladosporium cladosporioides</i> <i>Escherichia coli</i> <i>Kluyveromyces marxianus</i> <i>Lactobacillus plantarum</i> <i>Staphylococcus xylosus</i>			
<b>Analysis</b>	<b>Target</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target</b>	<b>N</b>	<b>F</b>	<b>X</b>
Aerobic micro-organism, 30 °C	All except <i>C. perfringens</i>	154	0%	3%	All	153	1%	1%	All	153	0%	1%
Psychrotrophic micro-organisms	All except <i>C. perfringens</i>	23	22%	0%	<i>B. thermosphacta</i>	23	0%	0%	All	23	9%	0%
Enterobacteriaceae	<i>(A. hydrophila)</i>	135	47%	0%	-	135	2%	0%	<i>E. coli</i>	135	1%	7%
<i>E. coli</i>	-	119	2%	0%	-	119	0%	0%	<i>E. coli</i>	118	2%	3%
Presumptive <i>B. cereus</i>	<i>(A. hydrophila)</i>	120	5%	0%	<i>B. cereus</i>	123	1%	2%	<i>B. cereus</i>	120	11%	4%
Coagulase-positive staphylococci	<i>S. aureus</i> <i>(S. warneri)</i>	110	3%	24%	-	109	6%	0%	<i>(S. xylosus)</i>	109	11%	0%
Lactic acid bacteria	<i>(S. aureus,</i> <i>S. warneri)</i>	54	28%	0%	-	54	46%	0%	<i>L. plantarum</i>	54	4%	2%
<i>C. perfringens</i>	<i>C. perfringens</i>	60	5%	7%	<i>C. perfringens</i>	60	5%	0%	-	58	2%	0%
Anaerobic sulphite-reducing bacteria	<i>C. perfringens</i>	68	1%	4%	<i>C. perfringens</i>	68	3%	3%	-	69	0%	0%
Aerobic microorg. in fish products	All except <i>C. perfringens</i>	38	0%	0%	All	38	0%	0%	All	38	0%	3%
H <sub>2</sub> S-prod. bacteria in fish products	<i>S. putrefaciens</i>	33	0%	0%	<i>S. putrefaciens</i>	32	9%	9%	-	32	0%	0%
Yeasts	-	131	2%	0%	<i>H. uvarum</i>	131	2%	5%	<i>K. marxianus</i>	130	1%	6%
Moulds	-	127	2%	0%	<i>A. flavus</i>	127	2%	4%	<i>C. cladosporioides</i>	125	8%	2%

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

## **Aerobic microorganisms 30 °C**

---

### **Sample A**

The strains of *A. hydrophila*, *S. warneri*, *S. aureus* and *S. putrefaciens* were target organisms. Three low and two high outliers were reported.

### **Sample B**

All strains in the sample were target organisms. One high outlier and one false negative result were reported.

The results were distributed with a main peak at  $\log_{10}$  4.0 cfu ml<sup>-1</sup> and a smaller peak around  $\log_{10}$  4.8 cfu ml<sup>-1</sup>. The presence of two peaks is likely due to whether *B. thermosphacta* has been detected or not. *B. thermosphacta* was present in approximately  $\log_{10}$  4.7 cfu ml<sup>-1</sup>, whereas the remaining microorganisms were present in concentrations below  $\log_{10}$  4.0 cfu ml<sup>-1</sup>.

The results in the higher peak could be attributed to the use of Petrifilm AC. *B. thermosphacta* is a psychrotrophic microorganism, but it can also grow at 30 °C. It is possible that the use of Petrifilm AC is more gentle to *B. thermosphacta* compared to the pour-plate method that is often used with PCA. It can also be noted that *B. thermosphacta* appears to have been detected at a concentration around  $\log_{10}$  4.7 cfu ml<sup>-1</sup> in the analysis of both psychrotrophic microorganisms and the analysis of aerobic microorganisms in fish and fish products. In both these analyses, incubation is done at temperatures lower than 30 °C.

### **Sample C**

All strains in the sample were target organisms. One low and one high outliers were reported.

### **General remarks**

As in previous proficiency testing rounds, most laboratories followed NMKL 86:2013 (29 %), 3M Petrifilm (21 %) and ISO 4833-1:2013 (20 %). The older NMKL 86:2006 and ISO 4833:2003 were still used by 8 % and 5 % of the laboratories, respectively. The different methods are however similar, and are all based on incubation on PCA or MCPA at 30 °C for 72 h. Users of Petrifilm AC can use a different time/temperature, depending on the method validation. 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.

The majority of the laboratories incubated on either PCA or Petrifilm AC. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method. A smaller number of laboratories used TEMPO AC, which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains different-sized wells. A substrate in the medium emits fluorescence with hydrolysed by the microorganisms. The number of microorganisms is determined by the number and size of the fluorescing wells.

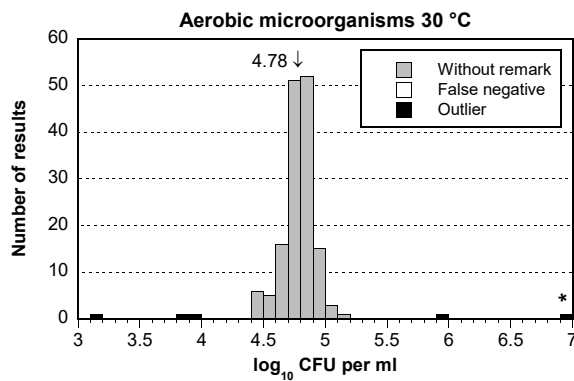


Results from analysis of aerobic microorganisms, 30 °C

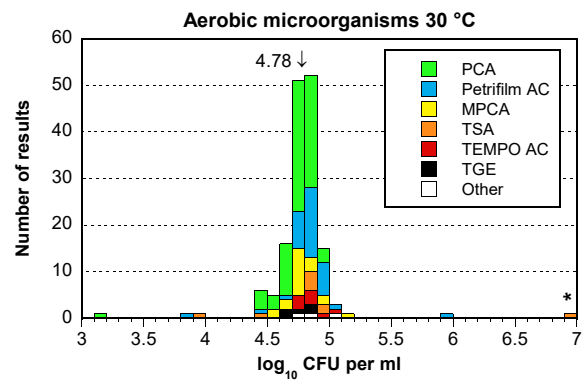
Medium	Sample A					Sample B					Sample C				
	N	n	m	s	F < >	N	n	m	s	F < >	N	n	m	s	F < >
All results	154	149	4.78	0.12	0 3 2	153	151	4.21	0.43	1 0 1	153	151	4.39	0.26	0 1 1
PCA	74	73	4.75	0.11	0 1 0	74	73	4.06	0.37	1 0 0	74	73	4.31	0.26	0 1 0
Petrifilm AC	35	33	4.83	0.11	0 1 1	35	35	4.69	0.32	0 0 0	35	35	4.55	0.23	0 0 0
MPCA	20	20	4.76	0.13	0 0 0	19	19	3.90	0.17	0 0 0	19	19	4.35	0.18	0 0 0
TSA	9	7	4.83	0.16	0 1 1	9	8	4.15	0.25	0 0 1	9	8	4.49	0.20	0 0 1
TEMPO AC	8	8	4.85	0.11	0 0 0	8	8	4.26	0.31	0 0 0	8	8	4.58	0.16	0 0 0
TGE	5	5	4.75	0.10	0 0 0	5	5	3.94	0.25	0 0 0	5	5	4.44	0.13	0 0 0
Other*	3	3	-	-	0 0 0	3	3	-	-	0 0 0	3	3	-	-	0 0 0

\* Other media includes BA and Compact Dry TC.

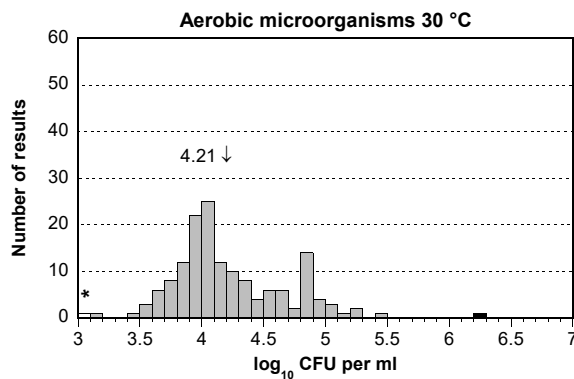
A



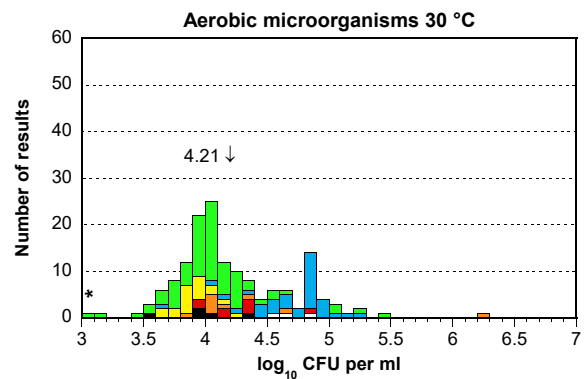
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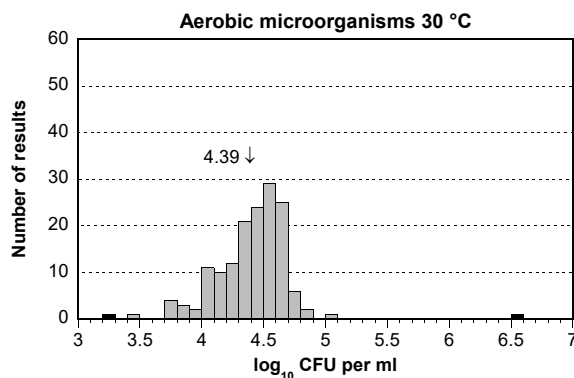
B



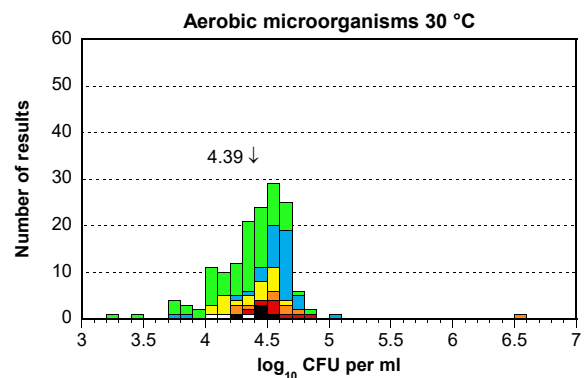
B



C



C



## **Psychrotrophic microorganisms**

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### **Sample A**

The strains of *A. hydrophila*, *S. warneri*, *S. aureus* and *S. putrefaciens* were target organisms. In the Swedish Food Agency's quality control a concentration of  $\log_{10}$  3.06 cfu ml<sup>-1</sup> was obtained after ten days incubation on PCA at 6.5 °C. The colonies that formed on the plates were small, and a magnifying lens was likely required by the laboratories during the enumeration.

Five laboratories reported a false negative result. Since only 18 laboratories reported a positive result, no outliers have been identified with statistical methods. Therefore, the median is also provided instead of the mean value in the tables in figures below.

### **Sample B**

*B. thermosphacta* was present in the highest concentration and was thus the main target organism. The sample also contained *B. cereus* and *S. putrefaciens*, in somewhat lower concentrations. These strains however grow less well compared to *B. thermosphacta* at low temperatures. The remaining microorganisms were present in considerably lower concentrations. In the Swedish Food Agency's quality control, a concentration of  $\log_{10}$  4.74 cfu ml<sup>-1</sup> was obtained after ten days incubation on PCA at 6.5 °C.

No outliers or false negative results were reported.

### **Sample C**

All strains in the sample were target organisms. In the Swedish Food Agency's quality control, a concentration of  $\log_{10}$  4.34 cfu ml<sup>-1</sup> was obtained after ten days incubation on PCA at 6.5 °C.

Two false negative results were reported.

### **General remarks**

In total, 23 laboratories performed the analysis. The majority of these (70 %) incubated on PCA, but MPCA (13 %) and Petrifilm AC (13 %) were also used. One laboratory incubated on Long & Hammer agar.

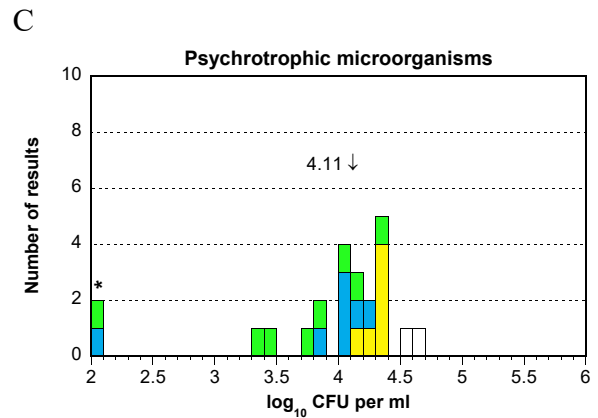
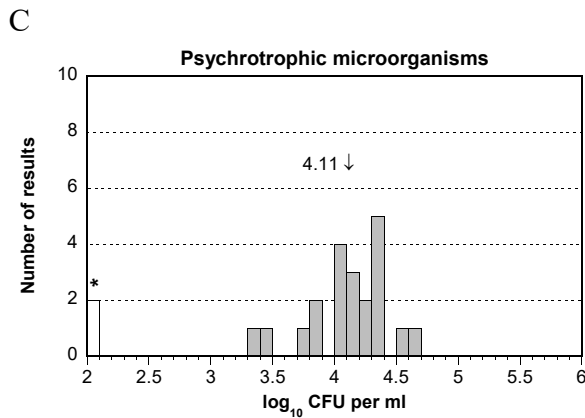
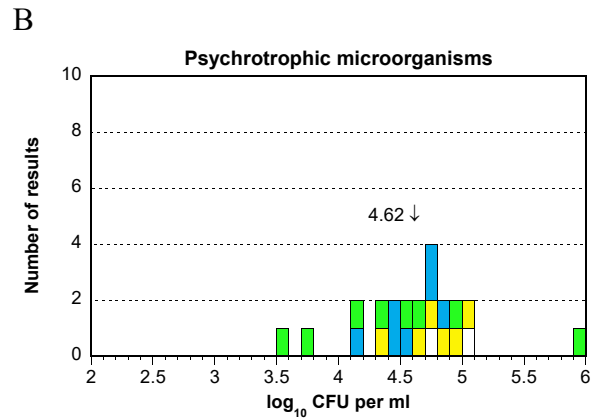
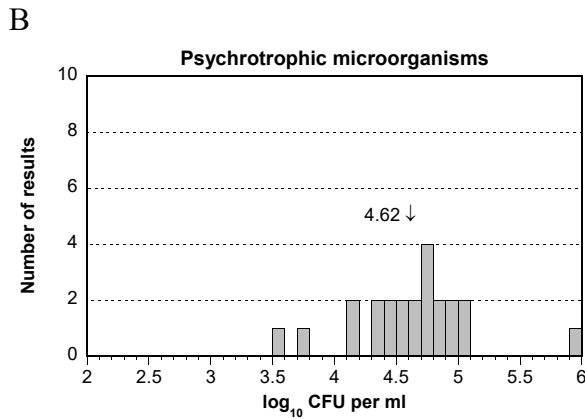
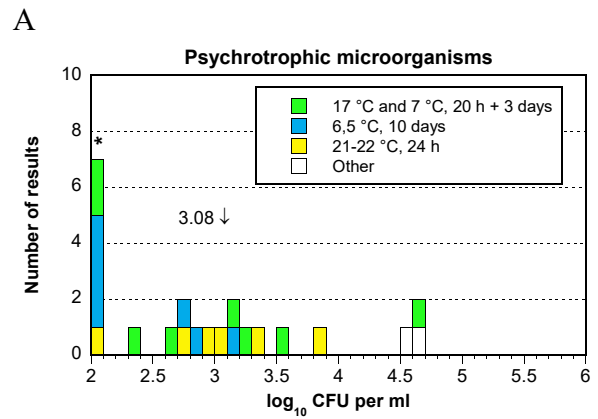
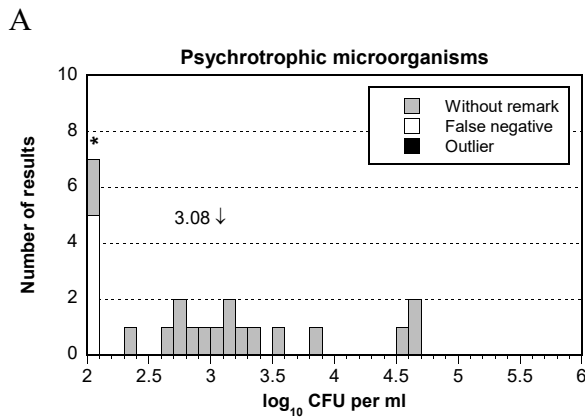
As before, there were considerable variations in the conditions for incubation, due to differences in the methods used by the laboratories. NMKL 86:2013 prescribes 10 days at 6.5 °C, but 20 h at 17 °C followed by 3 days at 7 °C can also be used. For enumeration of psychrotrophic microorganisms in milk, ISO 6730:2005/IDF 101:2005 stipulates incubation 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. Both of these were recently replaced by ISO 17410:2019, which stipulates 6.5 °C as the primary incubation temperature. Three laboratories followed NMKL 74:2000, which has been replaced by NMKL 86:2013.

The low number of participants makes it difficult to see if the false negative results are due to using a specific method or medium. The results are therefore difficult to evaluate, especially since a few laboratories also stated temperatures, incubation times or media that did not match the method they used. The majority of the methods could however be distributed into three groups. In general, 20-22 °C was used with 24 h incubation, and 6.5 °C with 10 days incubation. 17 °C / 7 °C was normally used with incubation for 20 h at 17 °C, followed by 3 days at 7 °C.

Results from analysis of psychrotrophic microorganisms

Method	Sample A						Sample B						Sample C					
	N	n	Med*	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >
All results	23	18	3.08	0.89	5	0 0	23	23	4.62	0.48	0 0 0	23	21	4.11	0.34	2	0 0	
17 and 7 °C, 20 h + 3 days	8	6	3.19	0.82	2	0 0	8	8	4.49	0.74	0 0 0	8	7	3.85	0.40	1	0 0	
6,5 °C, 10 days	7	5	2.79	0.74	2	0 0	7	7	4.56	0.25	0 0 0	7	6	4.04	0.14	1	0 0	
21-22 °C, 24 h	6	5	3.04	0.42	1	0 0	6	6	4.77	0.25	0 0 0	6	6	4.31	0.07	0	0 0	
Other	2	2	-	-	0	0 0	2	2	-	-	0 0 0	2	2	-	-	0	0 0	

\* Med = median



## **Enterobacteriaceae**

---

### **Sample A**

No target organism for the analysis was present in the sample. Despite this, 63 of 135 laboratories (47 %) reported a false positive result.

The strain of *A. hydrophila* is false positive for the analysis. In the Swedish Food Agency's quality control, it formed purple colonies on VRBG. *A. hydrophila* is however oxidase-positive, and can thus be distinguished from Enterobacteriaceae after confirmation with an oxidase test.

The false positive results could mainly be attributed to the use of Petrifilm EB and TEMPO EB, though many false positive results were also reported by laboratories that incubated on VRBG. False positive results were also more often reported by laboratories that did not perform a confirmation test.

### **Sample B**

No target organism for the analysis was present in the sample.

Three false positive results were reported.

### **Sample C**

The strain of *E. coli* was target organism and was present in approximately  $\log_{10}$  3.7 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control on VRBG, it formed typical dark red colonies surrounded by a bile salt precipitation zone. The strain was also oxidase-negative upon confirmation. No other colonies were observed on VRBG at the Swedish Food Agency.

Seven low and three high outliers were reported, as well as one false negative result.

### **General remarks**

Enterobacteriaceae are gram-negative and oxidase-negative bacteria that ferment glucose with the production of acid by-products. On VRBG they therefore 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.

As in previous proficiency testing rounds most laboratories followed either NMKL 144:2005 (43 %) or a method with Petrifilm EB (24 %), while the ISO methods (various versions) were used by 20 %. ISO 21528-2:2017 is based on colony-count, while 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 number of users of ISO 21528-2:2017 was higher compared to ISO 21528-2:2004 (10 % and 4 %, respectively). In comparison, six laboratories (4 %) stated the older ISO 21528-1:2004, while only one stated the new ISO 21528-1:2017.

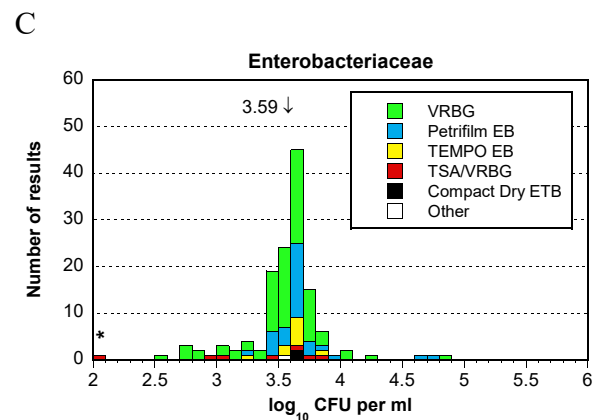
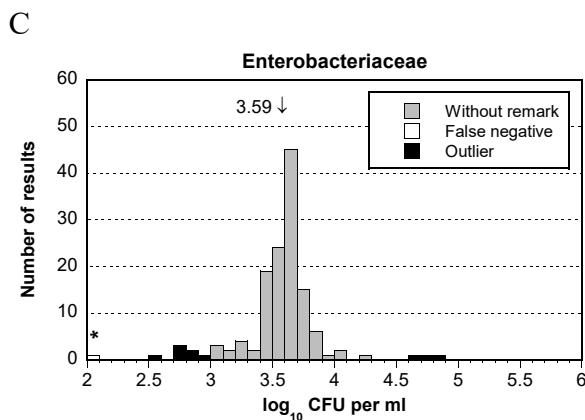
NMKL 144:2005 stipulates confirmation of presumptive colonies on VRBG with an oxidase test. ISO 21528-2:2017 stipulates confirmation of presumptive colonies with both an oxidase test and with a test for glucose fermentation. The majority of the laboratories that stated they performed a confirmation test specified that this consisted of an oxidase test.

With the exception of what is mentioned above regarding the false positive results for sample A, no clear differences in the results could be seen between the different

methods and media that were used. Somewhat higher results have often been noted for TEMPO EB in previous proficiency testing rounds, but this was not the case this time.

*Results from analysis of Enterobacteriaceae*

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	135	72	-	-	63	- -	135	132	-	-	3	- -	135	124	3.59	0.18	1	7	3
VRBG	83	54	-	-	29	- -	83	81	-	-	2	- -	82	75	3.58	0.20	0	6	1
Petrifilm EB	32	9	-	-	23	- -	32	32	-	-	0	- -	33	31	3.61	0.13	0	0	2
TEMPO EB	10	4	-	-	6	- -	10	10	-	-	0	- -	10	10	3.61	0.15	0	0	0
TSA/VRBG	7	4	-	-	3	- -	7	7	-	-	0	- -	7	5	3.54	0.29	1	1	0
Compact Dry ETB	2	1	-	-	1	- -	2	1	-	-	1	- -	2	2	-	-	0	0	0
Other	1	0	-	-	1	- -	1	1	-	-	0	- -	1	1	-	-	0	0	0



***Escherichia coli***

**Sample A**

No target organism for the analysis was present in the sample.  
Two false positive results were reported.

**Sample B**

No target organism for the analysis was present in the sample.  
No false positive results were reported.

**Sample C**

The strain of *E. coli* was target organism and was present in approximately  $\log_{10}$  3.7 cfu ml<sup>-1</sup> in the sample.

On TSA/VRB, the strain normally forms typical dark red colonies surrounded by a red precipitation zone. In the Swedish Food Agency’s quality control, no other colonies were observed on TSA/VRB. Upon confirmation, the strain formed both gas and indole in LTL SB. The strain was also positive for  $\beta$ -glucuronidase

One low and three high outliers were reported, as well as two false negative results.

## General remarks

As before, many laboratories used methods based on 3M™ Petrifilm™, and in total 30 % of the laboratories stated the use of “Petrifilm” as method. NMKL 125:2005 and ISO 16649-2:2001 were in comparison used by 32 % and 18 % of the laboratories, respectively. It should however be noted that some of the laboratories that followed NMKL 125:2005 and ISO 16649-2:2001 stated that they also incubated on Petrifilm EC/CC or Petrifilm SEC.

Among the less commonly used methods were ISO 7251:2005 and NMKL 96:2009. ISO 7251 is an MPN-based method for the detection *E. coli*. NMKL 96 is also based on MPN, and is adapted for analysis of coliform bacteria, thermotolerant coliform bacteria and *E. coli* in fish and seafood. One laboratory followed NMKL 164:2005 (*E. coli* O157), which is not correct. Further, this method is available in a revised version, NMKL 164:2019. It can also be mentioned that NMKL 125 is undergoing revision, and the new version will likely be more similar to ISO 16649-2.

The definition of *E. coli* differs between the methods. ISO 16649-2:2001 defines *E. coli* as bacteria that form typical blue colonies on 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 additional 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 these media facilitates detection of gas production due to lactose fermentation. In comparison, NMKL 125:2005 describes the analysis of both thermotolerant coliform bacteria and *E. coli*. Thermotolerant coliform bacteria are defined as those that form typical dark red colonies surrounded by a red precipitation zone on VRB after 24 h at 44 °C. Confirmation is by inoculation into either EC or LTLSB. In both these media, thermotolerant coliform bacteria produce gas as a result of lactose fermentation. *E. coli* are further defined as thermotolerant coliform bacteria that also produce indole in either LTLSB or tryptone broth.

In general, confirmation appears to have been performed when required by the method. For example, 88 % of the laboratories that followed NMKL 125:2005 performed a confirmation test. Confirmation was less often carried out by laboratories that used Petrifilm or that followed ISO 16649-2:2001, which is reasonable, since these methods do not require a confirmation. No obvious difference could however be seen between results from laboratories that confirmed, and those that did not. Laboratories that confirmed usually performed a test for production of either gas or indole.

As in previous proficiency testing rounds, several media were only used by a small number of laboratories. These have been placed together in the group “Other”. However as a whole, the results from the different media were very similar. The only notable difference was that the results for TBX were somewhat lower compared to other media. This has been observed also in several previous proficiency testing rounds, and is therefore considered normal.

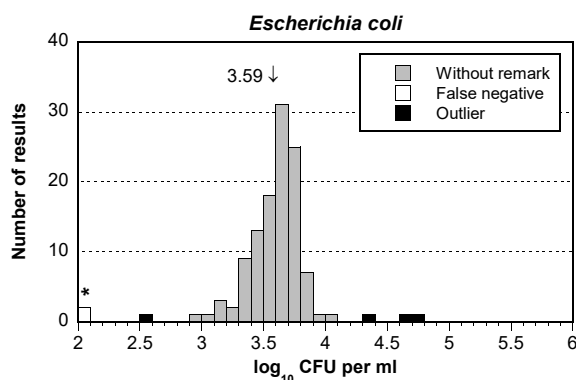
Incubation was somewhat more often done at 41.5-44 °C (56 %) than at 35-37 °C (42 %). Laboratories that incubated at the higher temperature range reported a total of seven outliers and false results, whereas those that incubated at the lower temperature only reported two false results. The mean values between the two temperature groups did however not differ.

### Results from analysis of *Escherichia coli*

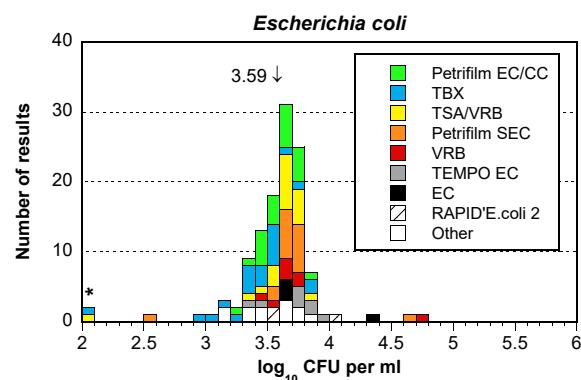
Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	119	117	-	-	2	- -	119	119	-	-	0	- -	118	112	3.59	0.19	2	1	3
Petrifilm EC/CC	23	22	-	-	1	- -	23	23	-	-	0	- -	23	23	3.58	0.14	0	0	0
TBX	23	22	-	-	1	- -	23	23	-	-	0	- -	22	21	3.46	0.24	1	0	0
TSA/VRB	20	20	-	-	0	- -	20	20	-	-	0	- -	20	19	3.63	0.12	1	0	0
Petrifilm SEC	17	17	-	-	0	- -	17	17	-	-	0	- -	18	16	3.68	0.08	0	1	1
VRB	8	8	-	-	0	- -	8	8	-	-	0	- -	8	7	3.66	0.11	0	0	1
TEMPO EC	8	8	-	-	0	- -	8	8	-	-	0	- -	8	8	3.69	0.21	0	0	0
EC	5	5	-	-	0	- -	5	5	-	-	0	- -	4	3	-	-	0	0	1
RAPID'E.coli 2	3	3	-	-	0	- -	3	3	-	-	0	- -	3	3	-	-	0	0	0
Other	12	12	-	-	0	- -	12	12	-	-	0	- -	12	12	3.52	0.24	0	0	0

\* The group Other includes Brilliance EC/CC, Compact Dry EC/CC, CHROMID® and Rebecca agar.

C



C



### Presumptive *Bacillus cereus*

#### Sample A

No target organism for the analysis was present in the sample. The strain of *A. hydrophila* is false positive for the analysis and was present in approximately log<sub>10</sub> 3.2 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control it formed grey-white colonies surrounded by a zone of haemolysis on BA. The strain could however be excluded after confirmation, since it does not display a precipitation zone on BcsA. Similarly, the strain of *S. aureus* may form atypical colonies on BA and BcsA. Six false positive results were reported. Five of these were from laboratories that followed NMKL 67, but do not appear to have performed a confirmation, something that is required by the method.

#### Sample B

The strain of *B. cereus* was target organism and was present in approximately log<sub>10</sub> 4.1 cfu ml<sup>-1</sup> in the sample. On BA it forms typical colonies surrounded by a zone of haemolysis. On BcsA it forms typical blue colonies surrounded by a precipitation zone. Two low and one high outlier were reported, as well as one false negative result.

### Sample C

The strain of *B. cereus* (not identical to the one in sample B) was target organism, but the strains of *E. coli*, *L. plantarum* and *S. xylosus* that were present in the sample may also form colonies on BA. The strain of *B. cereus* was present in approximately  $\log_{10}$  4.6 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control on BA, it formed typical colonies surrounded by a zone of haemolysis. On BcsA it formed typical blue colonies surrounded by a precipitation zone.

Four low and one high outlier were reported, as well as 13 false negative results. The false negative results were relatively evenly distributed between the methods and media that were used. An over-representation could however be seen for laboratories that stated they had only incubated on BcsA, and for laboratories that used Compact Dry X-BC.

### General remarks

Most laboratories followed either NMKL 67:2010 (54 %) or ISO 7932:2004 (23 %), which differ somewhat. NMKL 67:2010 is based on primary incubation on BA, and colonies are confirmed either on BcsA or on Cereus-Ident agar. On BA, *B. cereus* forms large, irregular grey colonies, surrounded by a distinct zone of haemolysis. On BcsA presumptive *B. cereus* form bluish colonies that are surrounded by a blue zone of precipitation, 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 is a result of *B. cereus* phosphatidylinositol phospholipase C (PI-PLC) cleavage of the chromogenic substrate X-myoinositol-1-phosphate. In contrast to the NMKL method, ISO 7932:2004 prescribes plating onto MYP. On MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity. The ISO method uses haemolysis on BA as the method for confirmation.

In addition to BA, BcsA and MYP, the chromogenic medium CBC was used by seven laboratories. Cleavage of the substrate X-Gluc present in CBC by *B. cereus*  $\beta$ -glucuronidase results in white colonies with a blue/green centre. Other media that were used to a lesser extent were Compact Dry X-BC, TEMPO BC, COMPASS® *Bacillus cereus* agar and BACARA™.

As in previous proficiency testing rounds the reporting of method data was in several cases ambiguous. For example, several laboratories reported combinations of method and media that are incompatible. As a general rule, this report shows the methods and media stated by the laboratories, regardless if these are compatible or not. Media that were only reported as "chromogenic medium" have been added to the group "Other". Despite these uncertainties, the results and mean values for the different methods and media were very similar. The exception was low results for Compact Dry X-BC in samples B and C. In addition, two of the five laboratories that used this medium also reported a false negative result for sample C.

An amendment was recently published for ISO 7932:2004 (Amd 1:2020). It contains optional tests, including for PCR detection of *cytK* genes. It can also be mentioned that NMKL 67 is scheduled for a minor revision.

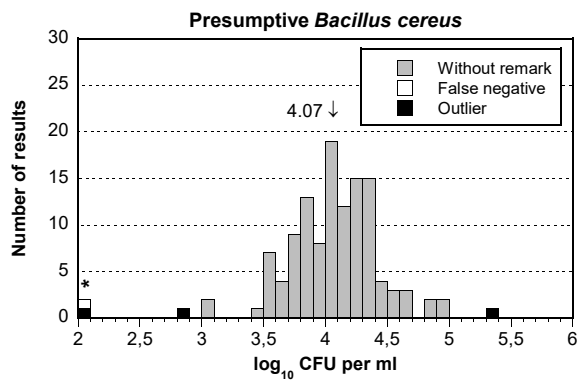


Results from analysis of presumptive *Bacillus cereus*

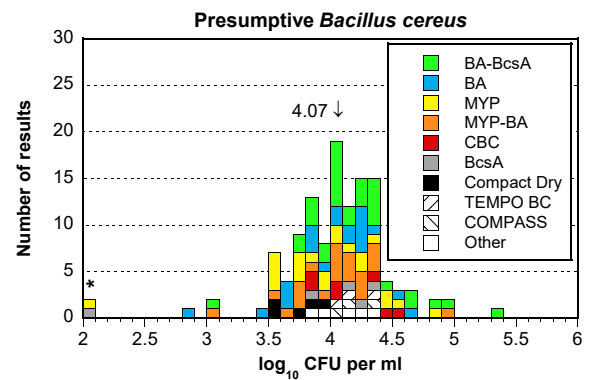
Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	120	114	-	-	6	-	-	123	119	4.07	0.33	1	2	1	120	102	4.29	0.27	13	4	1
BA-BcsA	31	31	-	-	0	-	-	31	30	4.15	0.36	0	0	1	29	26	4.38	0.21	2	0	1
BA	21	16	-	-	5	-	-	21	20	4.05	0.31	0	1	0	21	18	4.35	0.27	2	1	0
MYP	19	19	-	-	0	-	-	21	20	4.02	0.37	0	1	0	20	16	4.14	0.33	2	2	0
MYP-BA	22	22	-	-	0	-	-	22	22	4.02	0.36	0	0	0	22	22	4.38	0.20	0	0	0
CBC	6	6	-	-	0	-	-	7	7	4.16	0.27	0	0	0	7	7	4.33	0.12	0	0	0
BcsA	5	5	-	-	0	-	-	5	4	-	-	1	0	0	5	1	-	-	4	0	0
Compact Dry X-BC	5	4	-	-	1	-	-	5	5	-	-	0	0	0	5	2	-	-	2	1	0
TEMPO BC	3	3	-	-	0	-	-	3	3	-	-	0	0	0	3	3	-	-	0	0	0
COMPASS B. cereus	3	3	-	-	0	-	-	3	3	-	-	0	0	0	3	3	-	-	0	0	0
Other*	5	5	-	-	0	-	-	5	5	-	-	0	0	0	5	4	-	-	1	0	0

\* The group Other includes BACARA™.

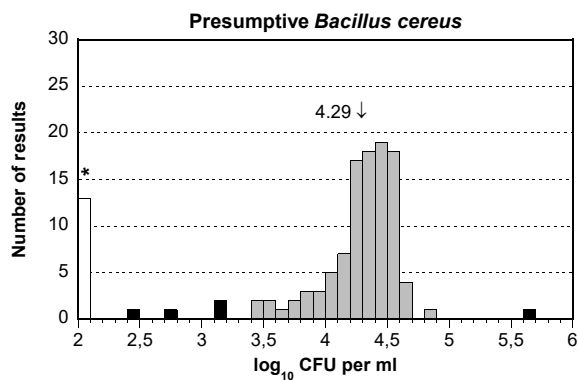
B



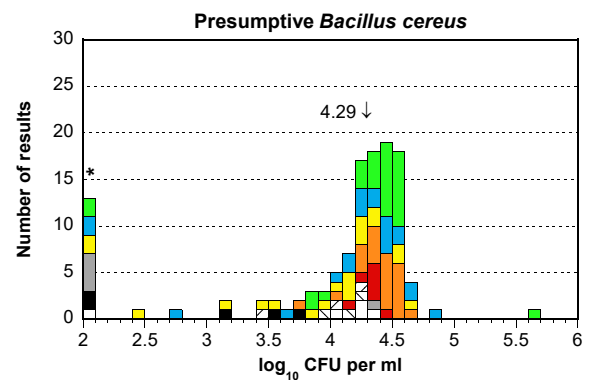
B



C



C



## Coagulase-positive staphylococci

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### Sample A

The strain of *S. aureus* was target organism, and was present in approximately  $\log_{10}$  4.2 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control on RPFA it formed typical dark grey colonies surrounded by a coagulase zone. The strain of *S. warneri* that was also present in the sample is false positive for the analysis. It is coagulase-negative and forms atypical colonies on RPFA, which are not surrounded by a coagulase zone.

Four low and 22 high outliers were reported, as well as three false negative results.

The high outliers are likely due to inclusion of *S. warneri*. The results were distributed with two peaks; a main peak corresponding to the concentration of *S. aureus* in the sample (approximately  $\log_{10}$  4.1 cfu ml<sup>-1</sup>) and a smaller peak corresponding to *S. warneri* (approximately  $\log_{10}$  4.7 cfu ml<sup>-1</sup>).

The high outliers could almost exclusively be attributed to the use of BP. Since the coagulase-activity is not tested on this medium, confirmation needs to be performed. In total, 20 of the 22 laboratories that reported high outliers also stated that they performed some kind of confirmation. Ten of these stated the confirmation consisted of a tube coagulase test, but a latex agglutination test was also fairly common (four laboratories).

### Sample B

No target organism for the analysis was present in the sample.

Seven false positive results were reported. The reported concentrations vary between  $\log_{10}$  1.78 and  $\log_{10}$  4.57 cfu ml<sup>-1</sup>, and it is therefore difficult to say which microorganism(s) that have been detected.

### Sample C

No target organism for the analysis was present in the sample. The coagulase-negative strain of *S. xylosus* is however false positive for the analysis, and was present in approximately  $\log_{10}$  3.3 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control it formed atypical colonies without a coagulase zone on RPFA.

Twelve false positive results were reported. Three of these were from laboratories that reported a false positive result also for sample B. The reported concentrations suggest that it is likely *S. xylosus* that has been detected. The twelve false positive results are therefore likely due to either a failed confirmation or not performing a confirmation (four laboratories).

### General remarks

Most laboratories (44 %) followed NMKL 66:2009. Other methods were 3M™ Petrifilm™ (14 %), ISO 6888-1:1999 (16 %) and ISO 6888-2:1999 (6 %). Both ISO 6888-1:1999 (based on BP) and ISO 6888-2:1999 (based on RPFA) were last reviewed by ISO in 2015 and remain current. An alternative confirmation by stab-culture in RPFA has however been added for ISO 6888-1 (ISO 6888-1:1999/Amd 2:2018). Three of the 18 laboratories that used ISO 6888-1 stated that they followed this amendment.

NMKL 66:2009 prescribes incubation on BP and/or RPFA. The colonies are confirmed by a positive result in a coagulase test. When using RPFA, the coagulase activity is instead tested directly in the medium. In comparison, ISO 6888-1:1999 stipulates

surface spreading on BP followed by confirmation with a coagulase test, whereas 6888-2:1999 stipulates the use of RPFA. On BP, *S. aureus* forms characteristic convex, shiny colonies that have a grey/black colour due to reduction of tellurite in the medium. The colonies are usually surrounded by a clear zone, due to proteolysis of egg yolk in the medium (lecithinase activity). An opaque halo may also form near the colony, due to precipitation caused by lipase activity. Petrifilm Staph is based on a modified Baird-Parker agar. It also contains a chromogenic indicator that causes *S. aureus* to form red/purple colonies.

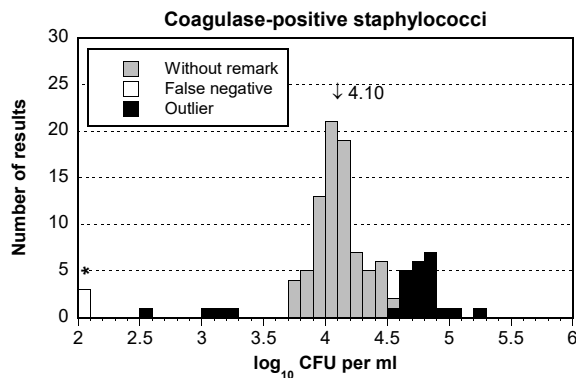
Taken together, the results were very similar for the most common media BP, RPFA and Petrifilm Staph, in all three samples. The exception was the high outliers in sample A that were reported primarily when using BP. Somewhat lower mean values have in previous proficiency testing rounds sometimes been seen for Petrifilm Staph, but this was as evident this time. Several media were used only by a small number of laboratories, which make them difficult to evaluate. Among these were EASY Staph®, TEMPO STA, Brilliance™ Staph 24, Compact Dry™ X-SA and RAPID'Staph.

In total, 72 % of the laboratories stated that they performed some kind of confirmation, usually a tube coagulase test. Traditionally, confirmation of coagulase-positive staphylococci is by detection of extracellular or bound coagulase (tube coagulase test and slide coagulase test respectively). Another common confirmation is a latex agglutination test. 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. Confirmation with Petrifilm Disk is based on detection of extracellular DNase, which is produced by the majority of coagulase-positive *S. aureus*, but also by the coagulase-positive staphylococci *S. intermedius* and *S. hyicus*. Toluidin blue O in the disks visualises DNase activity as a pink zone around the colonies.

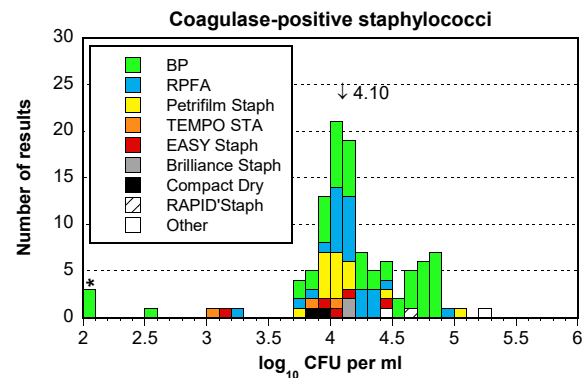
#### *Results from analysis of coagulase-positive staphylococci*

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	110	81	4.10	0.18	3	4	22	109	102	-	-	7	-	-	109	97	-	-	12	-	-
BP	53	31	4.11	0.19	3	1	18	53	51	-	-	2	-	-	53	46	-	-	7	-	-
RPFA	26	24	4.12	0.16	0	1	1	26	24	-	-	2	-	-	26	25	-	-	1	-	-
Petrifilm Staph	16	15	4.03	0.16	0	0	1	16	16	-	-	0	-	-	16	14	-	-	2	-	-
EASY Staph	5	4	-	-	0	1	0	5	4	-	-	1	-	-	5	4	-	-	1	-	-
TEMPO STA	3	2	-	-	0	1	0	3	3	-	-	0	-	-	3	3	-	-	0	-	-
Brilliance Staph 24	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	-	-
Compact Dry X-SA	2	2	-	-	0	0	0	2	0	-	-	2	-	-	2	2	-	-	0	-	-
RAPID'Staph	1	0	-	-	0	0	1	1	1	-	-	0	-	-	1	0	-	-	1	-	-
Other	2	1	-	-	0	0	1	1	1	-	-	0	-	-	1	1	-	-	0	-	-

A



A



## Lactic acid bacteria

### Sample A

No target organism for the analysis was present in the sample.

Fifteen false positive results were reported. The reported concentrations suggest that at least half of these are due to detection of either *S. aureus* and/or *S. warneri*, which may sometimes form small colonies on MRS and MRS-aB.

The majority of the false-positive results were reported by laboratories that incubated on MRS or MRS-aB. In comparison, no false positive results were reported by laboratories that incubated on Rogosa, or that used TEMPO LAB.

### Sample B

No target organism for the analysis was present in the sample.

Twenty-five false positive results were reported. These were distributed relatively evenly in an interval from  $\log_{10}$  0.18 cfu ml<sup>-1</sup> to  $\log_{10}$  5.18 cfu ml<sup>-1</sup>, and it is therefore difficult to find a clear-cut explanation to them. In the Swedish Food Agency's quality control, no colonies were detected on MRS-aB.

Confirmation does not appear to have had an impact on the outcome. As for sample A, most false positive results were reported by laboratories that incubated on MRS or MRS-aB. No false positive results were reported by laboratories that incubated on Rogosa, or that used TEMPO LAB.

### Sample C

The strain of *L. plantarum* was target organism and was present in approximately  $\log_{10}$  3.6 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's quality control it formed typical white colonies on MRS-aB. The strain is Gram-positive and catalase-negative. The strains of *B. cereus* and *S. xylosum* that are present in the sample may sometimes form small colonies on MRS-aB. They can however be excluded after confirmation with a catalase test.

One high outlier and two false negative results were reported.

### General remarks

The majority of the laboratories stated that they followed NMKL 140, either NMKL 140:2007 (39 %), or the older NMKL 140:1991 (13 %). The older method prescribes

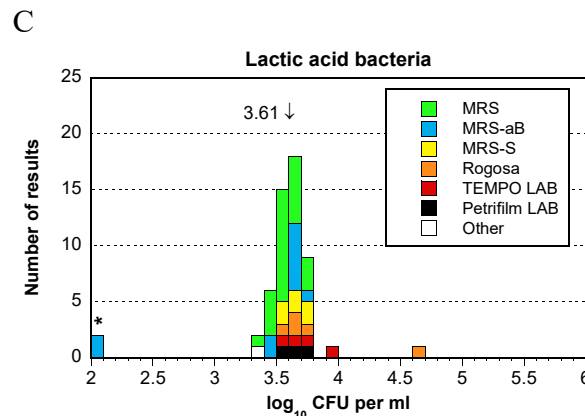
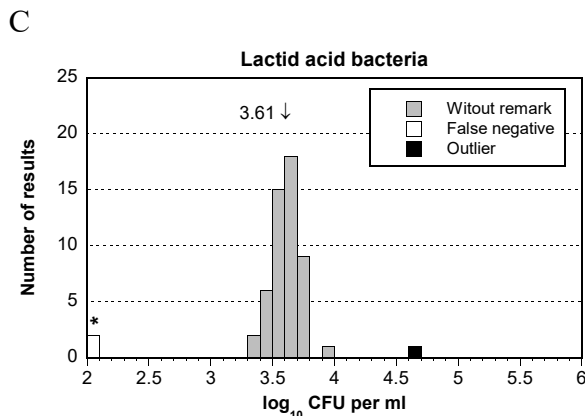
spreading onto MRS-S, whereas the new method prescribes MRS-aB. In comparison, ISO 15214:1998, which was used by 13 % of the laboratories, uses a pour-plate method with MRS. On both MRS-S and MRS-aB, lactic acid bacteria normally form 1.5-2 mm large grey-white colonies. On Petrifilm LAB, lactic acid bacteria form red colonies. The plates also facilitate distinction between gas producing (heterofermentative) and non-gas producing (homofermentative) lactic acid bacteria. ISO 15214:1998 was reviewed by ISO in 2015, and remains current. NMKL 140 is however considered for revision, and changes will likely be made to the confirmation tests. Two laboratories stated ISO 7889 / IDF 117, which is a method for characteristic microorganisms in yoghurt at 37 °C.

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) 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. Possibly, this might have contributed to the false positive results for MRS-aB in samples A and B. It can also be noted that two of the three laboratories that used Petrifilm LAB reported a false positive result for both samples A and B.

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 roughly half (54 %) of the laboratories. Usually, it consisted of a catalase test, but Gram staining was also common. As a whole, the use of a confirmation test does not appear to have had an impact on the result. The results with an annotation were also distributed proportionally between laboratories that performed a confirmation test, and those that did not. This was true for all three samples.

*Results from analysis of lactic acid bacteria*

Medium	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	54	39	-	-	15	- -	54	29	-	-	25	- -	54	51	3.61	0.11	2	0	1
MRS	24	17	-	-	7	- -	24	11	-	-	13	- -	24	24	3.58	0.10	0	0	0
MRS-aB	11	5	-	-	6	- -	11	4	-	-	7	- -	11	9	3.62	0.11	2	0	0
MRS-S	6	6	-	-	0	- -	6	4	-	-	2	- -	6	6	3.64	0.07	0	0	0
Rogosa	5	5	-	-	0	- -	5	5	-	-	0	- -	5	4	-	-	0	0	1
TEMPO LAB	4	4	-	-	0	- -	4	4	-	-	0	- -	4	4	-	-	0	0	0
Petrifilm LAB	3	1	-	-	2	- -	3	1	-	-	2	- -	3	3	-	-	0	0	0
Other	1	1	-	-	0	- -	1	0	-	-	1	- -	1	1	-	-	0	0	0



## *Clostridium perfringens*

### Sample A

The strain of *C. perfringens* was target organism and was present in approximately  $\log_{10}$  3.1 cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's control it formed black colonies on TSC. The strain is non-motile and ferments lactose.

Three low and one high outlier were reported, as well as three false negative results.

### Sample B

The strain of *C. perfringens* (identical to the one in sample A) was target organism and was present in approximately  $\log_{10}$  2.6 cfu ml<sup>-1</sup> in the sample.

Three false negative results were reported.

### Sample C

No target organism for the analysis was present in the sample.

One false positive result was reported.

### General remarks

The majority of the laboratories (67 %) followed NMKL 95:2009. One laboratory followed the older NMKL 95:2006. ISO 7937:2004 was followed by 25 % of the laboratories. One laboratory stated that they analysed according to NMKL 56:2015 (Sulphite-reducing Clostridia). This method includes detection of *C. perfringens* by referring to the confirmation tests in NMKL 95. ISO 7937:2004 was 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 obvious differences could be seen in the results from the different methods.

ISO 7937:2004 prescribes a pour-plate method with TSC, while NMKL 95 prescribes surface-spreading on mCP and/or pour-plating with TSC. Here, the majority (90 %) of the laboratories reported the use of TSC. On TSC, *C. perfringens* form black colonies after anaerobic incubation at 37 °C. In addition to TSC – mCP, SC, JSA and SPS were used by one laboratory each. Due to the low number of users, comparisons with TSC are difficult to make. The laboratory that incubated on SPS did report a false negative result for sample A and a false positive result for sample C, but this is likely due to mix-

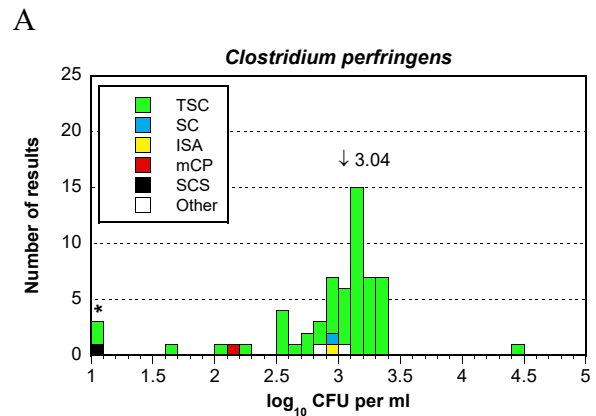
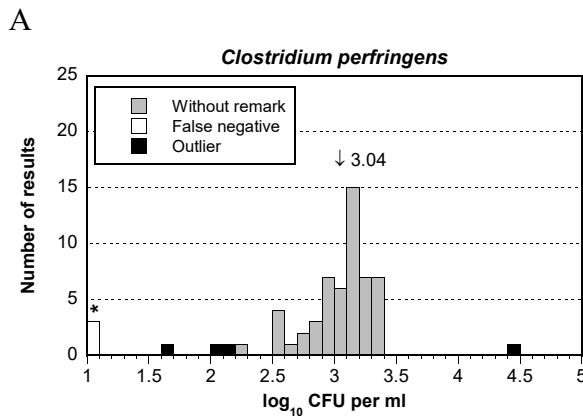
up of the samples during the analysis. It could here also be mentioned two studies that recommend TSC for the analysis of *C. perfringens* in food samples (2, 3).

With NMKL 95:2009 suspected and typical colonies are confirmed with a motility test and a test for lactose fermentation. *C. perfringens* is non-motile and forms acid and gas as a consequence of lactose fermentation. The method for confirmation is similar in ISO 7937:2004. *C. perfringens* can also be confirmed since it forms a double haemolytic zone upon anaerobic incubation on BA. In total, 93 % of the laboratories stated they performed some kind of confirmation. Common confirmation methods were motility test, test for lactose fermentation, test for haemolysis on BA, and test for absence of growth in aerobic conditions.

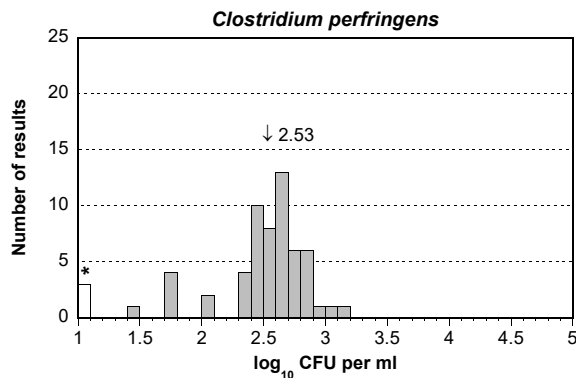
The majority of the laboratories (93 %) incubated at 37 °C. Three laboratories (5 %) incubated at 44 °C and one laboratory at 30 °C. *C. perfringens* normally grows at both 37 °C and 44 °C and even though only few laboratories incubated at 44 °C, the temperature does not appear to have had an effect on the outcome.

*Results from analysis of Clostridium perfringens*

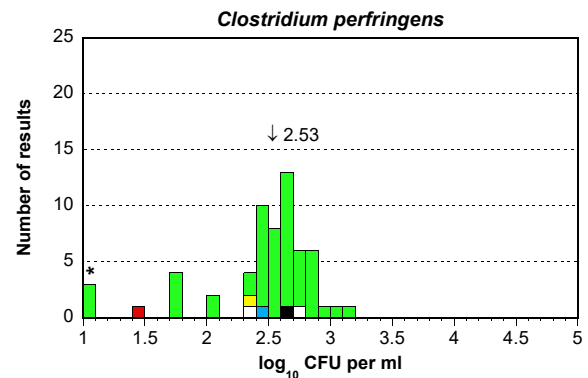
Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	60	53	3.04	0.24	3	3	1	60	57	2.53	0.33	3	0	0	58	57	-	-	1	-	-
TSC	54	49	3.05	0.25	2	2	1	54	51	2.55	0.31	3	0	0	52	52	-	-	0	-	-
SC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	-	-
JSA	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	-	-
mCP	1	0	-	-	0	1	0	1	1	-	-	0	0	0	1	1	-	-	0	-	-
SPS	1	0	-	-	1	0	0	1	1	-	-	0	0	0	1	0	-	-	1	-	-
Other	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-



B



B



## Anaerobic sulphite-reducing bacteria

### Sample A

The strain of *C. perfringens* was target organism and was present in approximately  $\log_{10} 3.1$  cfu ml<sup>-1</sup> in the sample. In the Swedish Food Agency's control it formed black colonies on ISA. The black colour was less distinct after 48 hours, compared to after 24 hours. Low results may therefore be a consequence of only recording results after 48 hours.

One low and two high outliers were reported, as well as one false negative result.

### Sample B

The strain of *C. perfringens* (identical to the one in sample A) was target organism and was present in approximately  $\log_{10} 2.6$  cfu ml<sup>-1</sup> in the sample.

One low and one high outlier were reported, as well as two false negative results.

### Sample C

No target organism for the analysis was present in the sample.

No false positive results were reported.

### General remarks

As in previous proficiency testing rounds, the majority of the laboratories followed a version of NMKL 56. The proportion of users of the new NMKL 56:2015 was somewhat higher than previously, and it was now used by 16 % of the laboratories. Most laboratories however still followed either NMKL 56:2008 (47 %) or the considerably older NMKL 56:1994 (6 %). In comparison, ISO 15213:2003 was used 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. Three 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"). No obvious differences in results between the methods could however be identified.

Both NMKL 56:2015 and ISO 15213:2003 prescribe pour-plate methods with ISA, which was also the medium most frequently used by the laboratories. With ISA, black

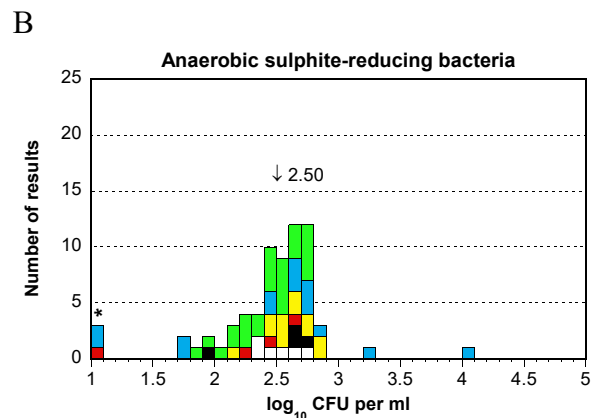
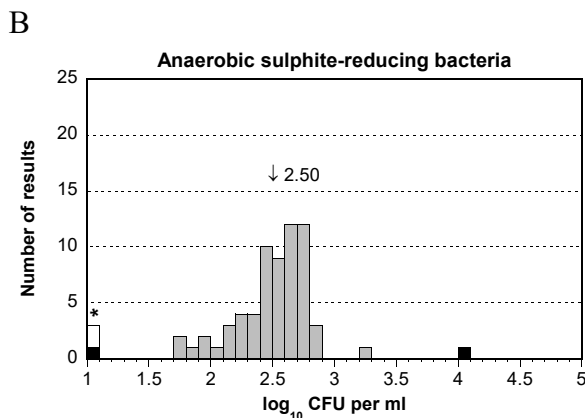
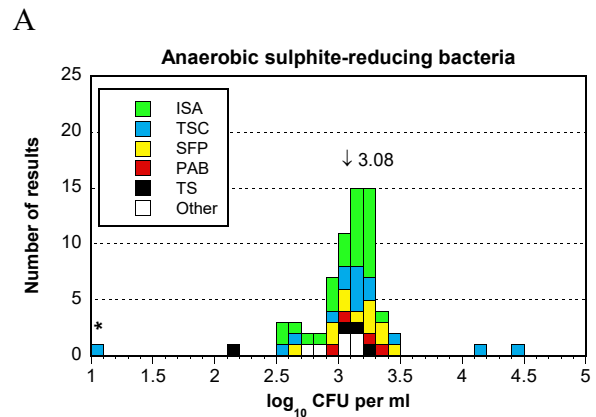
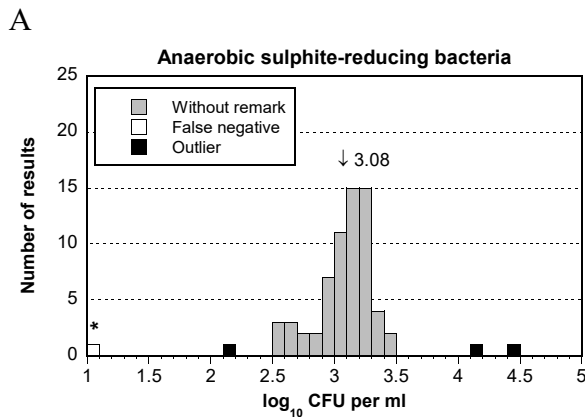


colonies (possibly surrounded by a black zone) are considered as sulphite-reducing bacteria. The black colour of the colonies comes from iron sulphide, which is formed as a precipitate of  $\text{Fe}^{3+}$  in the medium, and  $\text{H}_2\text{S}$  that is produced by the reduction of sulphite. Growth 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 TSC, SFP, PAB and 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. Use of these media did not cause any obvious problems here. Though relatively many outliers and false negative results were reported for samples A and B by laboratories that used TSC, the number of users was low, and it can therefore not be ruled out that this was simply due to chance.

*Results from analysis of anaerobic sulphite-reducing bacteria*

Substrat	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	68	64	3.08	0.21	1	1 2	68	64	2.50	0.28	2	1 1	69	69	-	-	0	-	-
ISA	27	27	3.05	0.22	0	0 0	27	27	2.44	0.26	0	0 0	28	28	-	-	0	-	-
TSC	15	12	3.07	0.25	1	0 2	15	12	2.55	0.41	1	1 1	15	15	-	-	0	-	-
SFP	12	12	3.14	0.21	0	0 0	12	12	2.59	0.18	0	0 0	12	12	-	-	0	-	-
PAB	4	4	-	-	0	0 0	4	3	-	-	1	0 0	4	4	-	-	0	-	-
TS	4	3	-	-	0	1 0	4	4	-	-	0	0 0	4	4	-	-	0	-	-
Other	6	6	3.01	0.15	0	0 0	6	6	2.50	0.16	0	0 0	6	6	-	-	0	-	-



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

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### Sample A

The strains of *A. hydrophila*, *S. warneri*, *S. aureus* and *S. putrefaciens* were target organisms.

No outliers or false negative results were reported.

### Sample B

All strains in the sample were target organisms.

No outliers or false negative results were reported.

### Sample C

The strains of *B. cereus*, *L. plantarum*, *E. coli* and *S. xylosus* were target organisms.

One low outlier was reported.

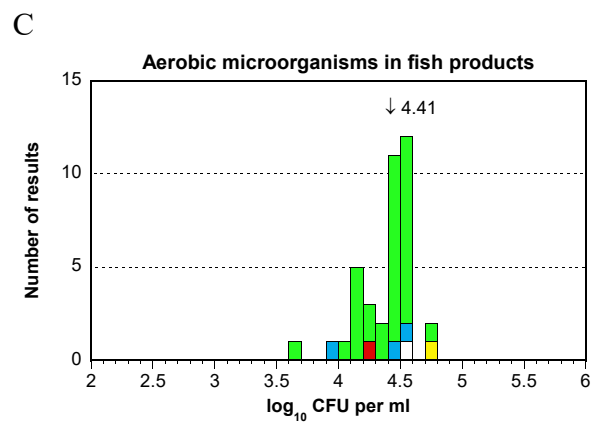
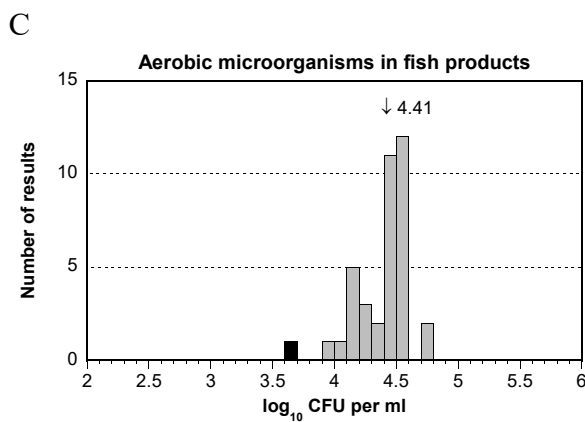
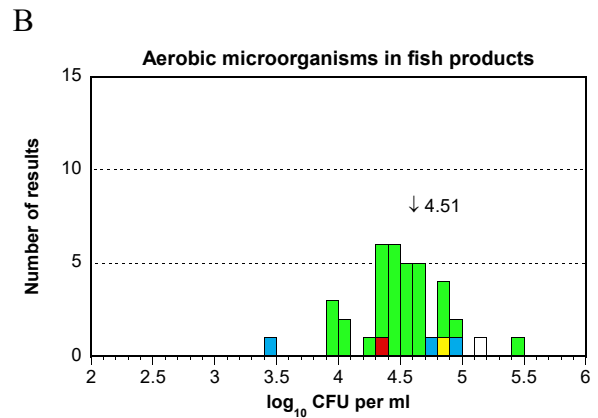
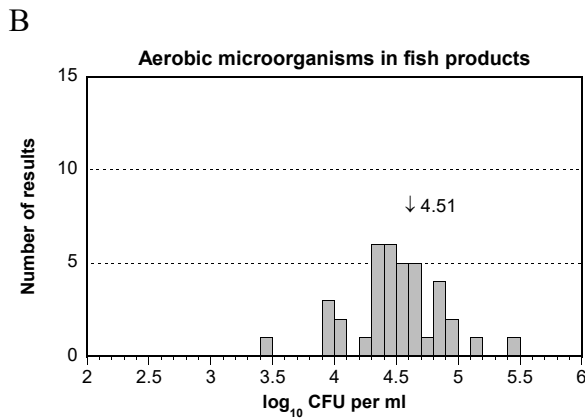
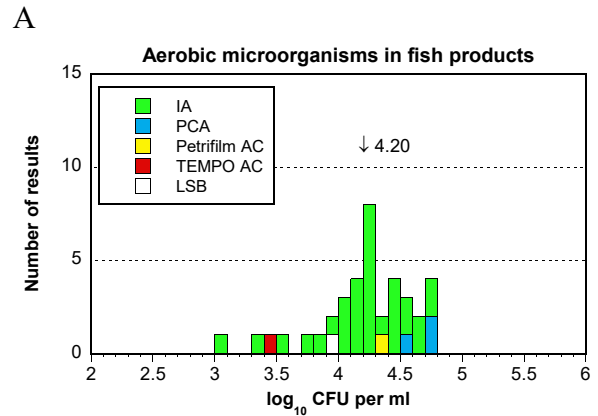
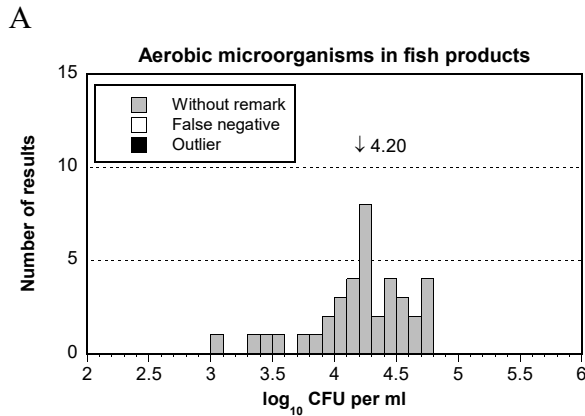
### General remarks

The majority of the laboratories (84 %) followed the method for aerobic microorganisms and specific spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with IA, which was consequently the medium most frequently used by the laboratories (84 %). Two laboratories followed NMKL 86 ("Aerobic microorganisms in food") and thus incubated on PCA. Though this method is adapted for use in all types of food, it also refers to NMKL 184:2006 for analysis of fish and fish products. One laboratory followed ISO 4833-1:2013 ("Aerobic microorganisms"). Yet another laboratory followed NMKL 96:2003, which uses the same method for total aerobic count as NMKL 184:2006. This laboratory however incubated in LSB, which is not correct. NMKL 96:2003 has also 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.

### Results from analysis of aerobic microorganisms in fish products, 20-25 °C

Substrat	Sample A						Sample B						Sample C								
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >			
All results	38	38	4.20	0.41	0	0	0	38	38	4.51	0.37	0	0	0	38	37	4.41	0.18	0	1	0
IA	32	32	4.18	0.38	0	0	0	32	32	4.49	0.31	0	0	0	32	31	4.41	0.17	0	1	0
PCA	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
Petrifilm AC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
TEMPO AC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
LSB	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0



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

### Sample A

The strain of *S. putrefaciens* was target organism for the analysis. In the Swedish Food Agency's control it formed black colonies on IA, with a concentration of approximately  $\log_{10} 4.1 \text{ cfu ml}^{-1}$ .

No outliers or false negative results were reported.

## Sample B

The strain of *S. putrefaciens* (identical to the one in sample A) was target organism for the analysis. In the Swedish Food Agency's control on IA, the concentration was approximately  $\log_{10} 3.6 \text{ cfu ml}^{-1}$ .

Three low outliers were reported, as well as three false negative results.

## Sample C

No target organism for the analysis was present in the sample. In the Swedish Food Agency's quality control, only white colonies were observed on IA.

No false positive results were reported.

## General remarks

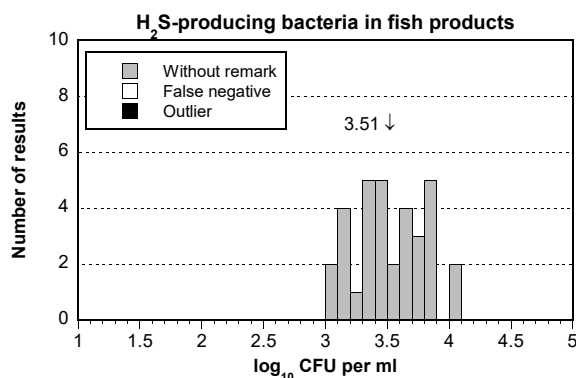
The majority of the laboratories (97 %) followed the method for aerobic micro-organisms and specific spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with IA, which was consequently the medium most frequently used by the laboratories (97 %). One laboratory followed NMKL 96:2003 ("Bacterial examinations in fresh and frozen seafood"), which includes the analysis of  $\text{H}_2\text{S}$ -producing bacteria. This laboratory however incubated in LSB, which is not correct. NMKL 96:2003 has also been replaced by NMKL 96:2009 which refers to NMKL 184:2006 for the analysis of total aerobic count and specific spoilage organisms in fish and seafood.

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

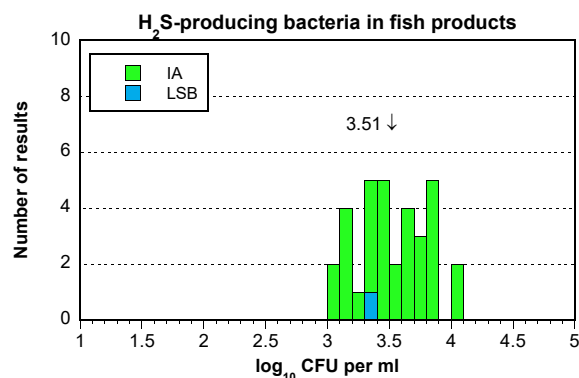
### Results from analysis of $\text{H}_2\text{S}$ -producing bacteria in fish products

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	33	33	3.51	0.29	0	0	0	32	26	2.83	0.42	3	3	0	32	32	-	-	0	-	-
IA	32	32	3.52	0.29	0	0	0	31	25	2.85	0.42	3	3	0	31	31	-	-	0	-	-
LSB	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	-	-

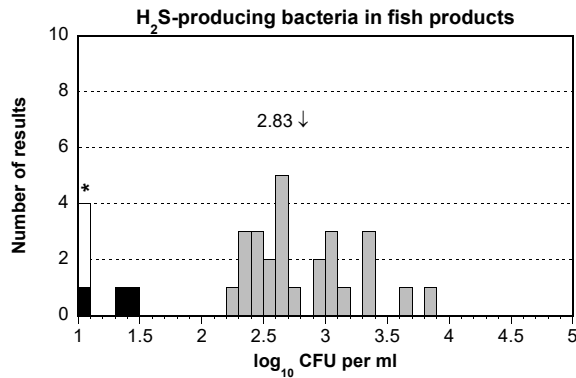
A



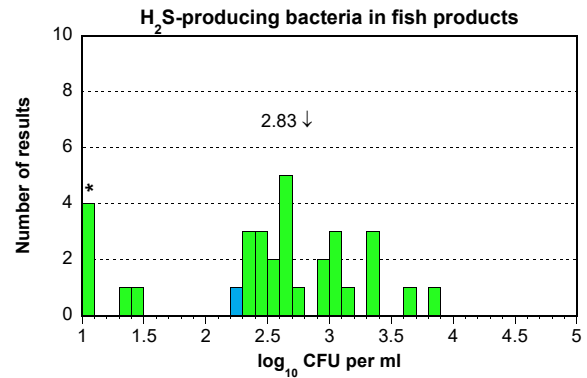
A



B



B



## Yeasts and moulds

### Sample A

No target organism for the analysis was present in the sample, neither for yeasts nor for moulds.

Two and three false positive results were reported for the analysis of yeasts and of moulds, respectively.

### Sample B

The strain of *H. uvarum* was target organism for the analysis of yeasts and the strain of *A. flavus* for the analysis of moulds. They were present in approximately log<sub>10</sub> 2.4 and log<sub>10</sub> 2.3 cfu ml<sup>-1</sup>, respectively.

In the analysis of yeasts, two low and five high outliers were reported, as well as three false negative results.

In the analysis of moulds, one low and four high outliers were reported, as well as two false negative results.

### Sample C

The strain of *K. marxianus* was target organism for the analysis of yeasts and the strain of *C. cladosporioides* for the analysis of moulds. They were present in approximately log<sub>10</sub> 2.5 and log<sub>10</sub> 2.2 cfu ml<sup>-1</sup>, respectively.

In the Swedish Food Agency's quality control *K. marxianus* formed shiny milk white colonies on DG18 and shiny pink colonies on DRBC. *C. cladosporioides* formed typical dark green colonies on both DG18 and DRBC.

In the analysis of yeasts, two low and six high outliers were reported, as well as one false negative result.

In the analysis of moulds, one low and one high outliers were reported, as well as ten false negative results. The false negative results could not be attributed to a specific method or medium.

### General remarks

In principle, the same laboratories analysed yeasts and moulds, and they generally used identical methods for both analyses. The methods mainly consisted of NMKL 98:2005 and ISO 6611:2004 / IDF 94:2004, but 3M™ Petrifilm™ and ISO 21527-1:2008 /

ISO 21527-2:2008 were also commonly used. Two laboratories stated that they 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 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 either OGYE or 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 food with  $a_w > 0,95$  (e.g. fruit, vegetables, meat and milk products) while DG18 is recommended for food with  $a_w \leq 0,95$  (e.g. dried fruit, dried meat, grains and nuts). OGYE is recommended if only yeasts are to be analysed.

Outliers and false results were relatively evenly distributed between the main methods and media that were used. The mean values of the different groups were also similar. Several methods and media were however used by only a small number of laboratories. It is therefore difficult to make certain conclusions on potential differences in the results for these.

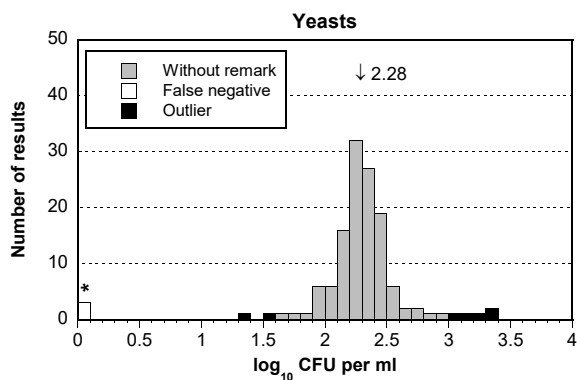
Four laboratories stated that they used TEMPO YM, sometimes in combination with other methods/media. The results from these laboratories have been included in the evaluation, but in some cases they have likely been determined as outliers or false results since the method in TEMPO YM gives a combined value for yeasts and moulds. 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*

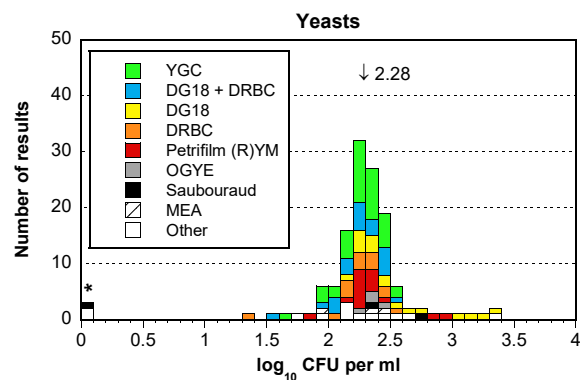
Medium	Sample A						Sample B						Sample C							
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >		
All results	131	129	-	-	2	-	131	121	2.28	0.20	3	2	5	130	121	2.25	0.15	1	2	6
YGC	39	39	-	-	0	-	39	39	2.24	0.18	0	0	0	38	36	2.26	0.14	1	1	0
DG18 + DRBC	22	22	-	-	0	-	22	21	2.26	0.17	0	1	0	21	20	2.24	0.14	0	1	0
DG18	17	17	-	-	0	-	17	13	2.38	0.18	0	0	4	17	14	2.20	0.15	0	0	3
DRBC	14	13	-	-	1	-	14	13	2.27	0.14	0	1	0	14	14	2.25	0.16	0	0	0
Petrifilm YM/RYM	16	16	-	-	0	-	16	16	2.34	0.24	0	0	0	16	16	2.27	0.14	0	0	0
OGYE	5	5	-	-	0	-	4	4	-	-	0	0	0	5	5	-	-	0	0	0
Saubouraud	3	2	-	-	1	-	3	2	-	-	1	0	0	3	2	-	-	0	0	1
MEA	3	3	-	-	0	-	3	3	-	-	0	0	0	3	1	-	-	0	0	2
Other*	12	12	-	-	0	-	13	10	2.24	0.27	2	0	1	13	13	2.28	0.20	0	0	0

\* Other media includes Compact Dry YM, PDA and TEMPO YM.

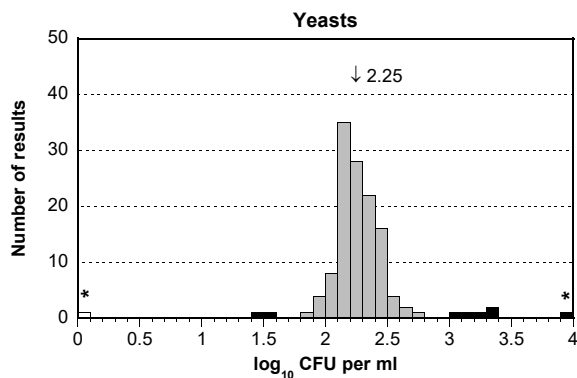
B



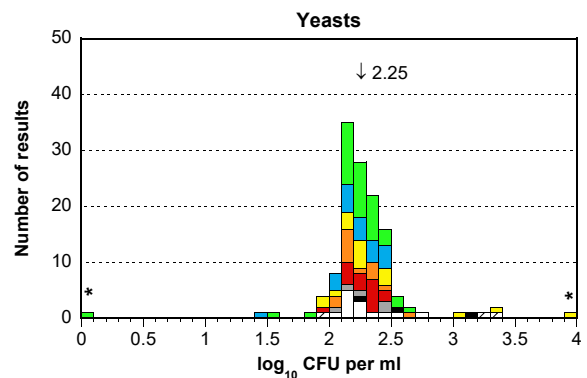
B



C



C

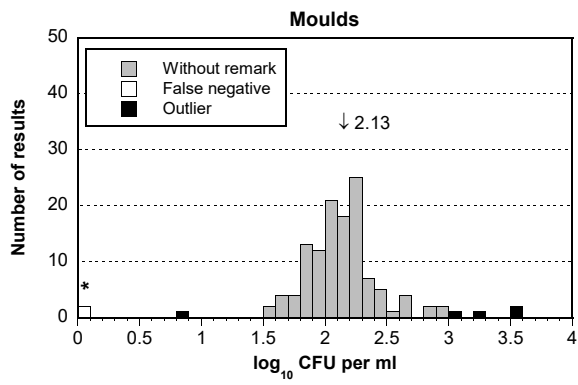


### Results from analysis of moulds

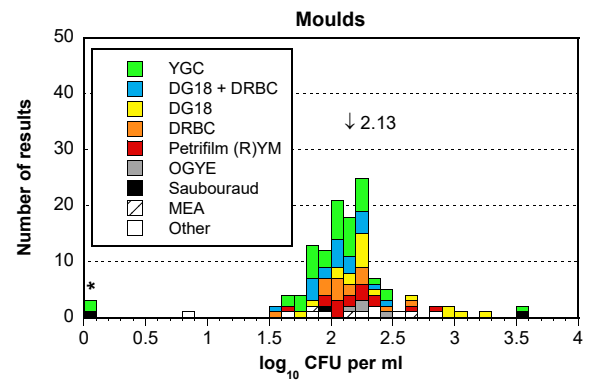
Medium	Sample A						Sample B						Sample C					
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >
All results	127	124	-	-	3	- -	127	120	2.13	0.27	2	1 4	125	113	1.84	0.28	10	1 1
YGC	39	39	-	-	0	- -	39	37	2.04	0.20	1	0 1	38	35	1.72	0.31	3	0 0
DG18 + DRBC	20	20	-	-	0	- -	21	21	2.07	0.21	0	0 0	19	18	1.72	0.21	1	0 0
DG18	18	18	-	-	0	- -	18	16	2.26	0.34	0	0 2	18	16	2.01	0.28	1	0 1
DRBC	15	14	-	-	1	- -	15	15	2.11	0.25	0	0 0	15	15	1.89	0.25	0	0 0
Petrifilm YM/RYM	15	15	-	-	0	- -	15	15	2.20	0.30	0	0 0	15	13	1.83	0.16	2	0 0
OGYE	5	5	-	-	0	- -	4	4	-	0.11	0	0 0	5	5	-	-	0	0 0
Saubouraud	3	2	-	-	1	- -	3	1	-	-	1	0 1	3	3	-	-	0	0 0
MEA	3	3	-	-	0	- -	3	3	-	-	0	0 0	3	3	-	-	0	0 0
Other*	9	8	-	-	1	- -	9	8	2.22	0.41	0	1 0	9	5	1.87	0.27	3	1 0

\* Other media includes Compact Dry YM, PDA and TEMPO YM.

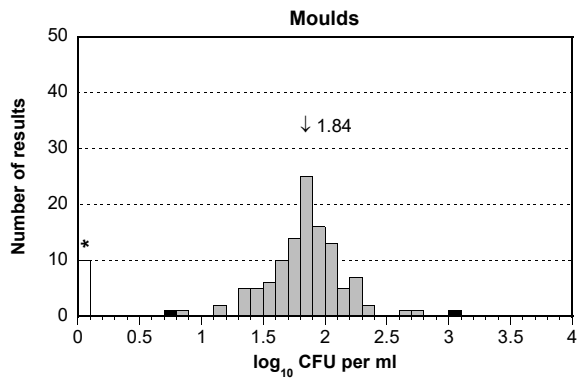
B



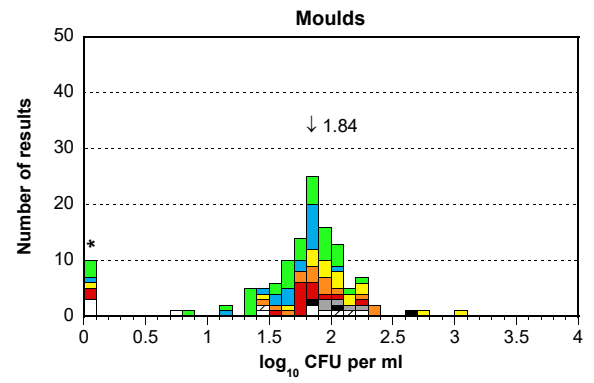
B



C



C





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

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### **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 (2). 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**

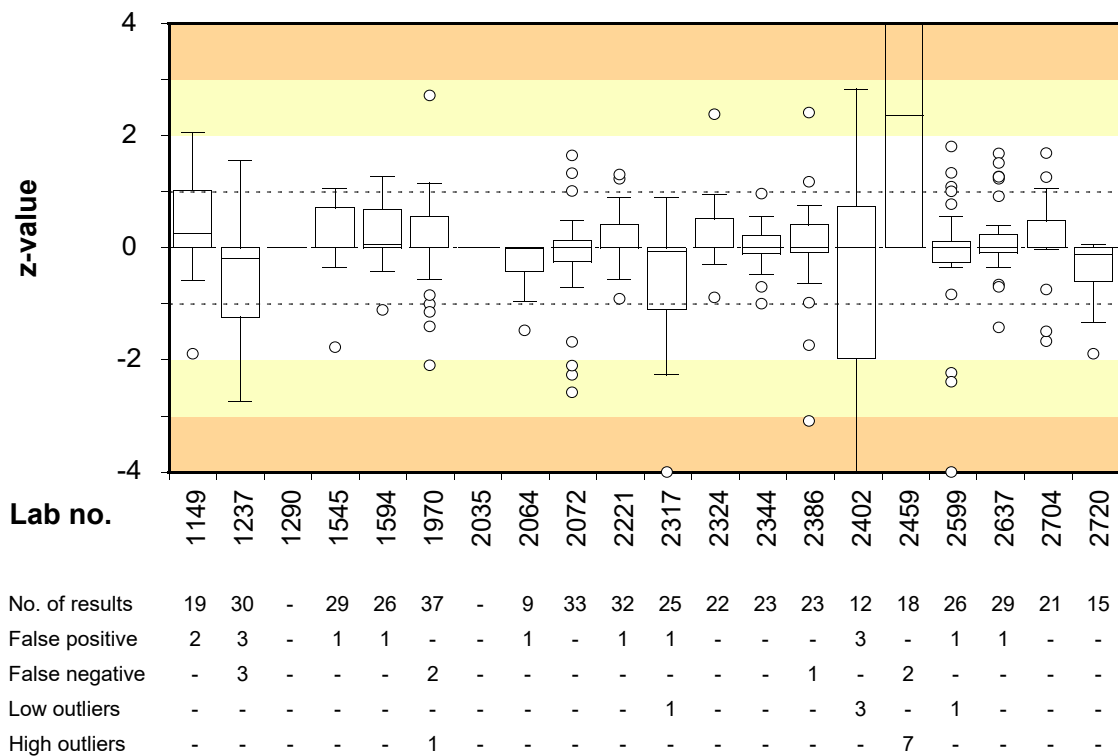
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

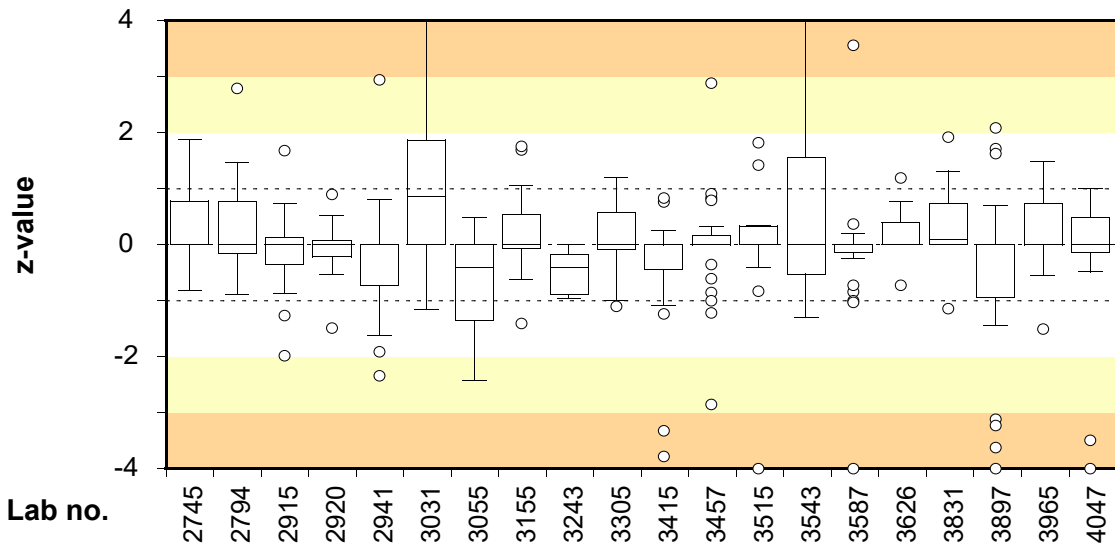
The box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the achievement of each laboratory. A small box, centred around zero, indicates that the results of the individual laboratory, with false results excluded, are close to the general mean values calculated for all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

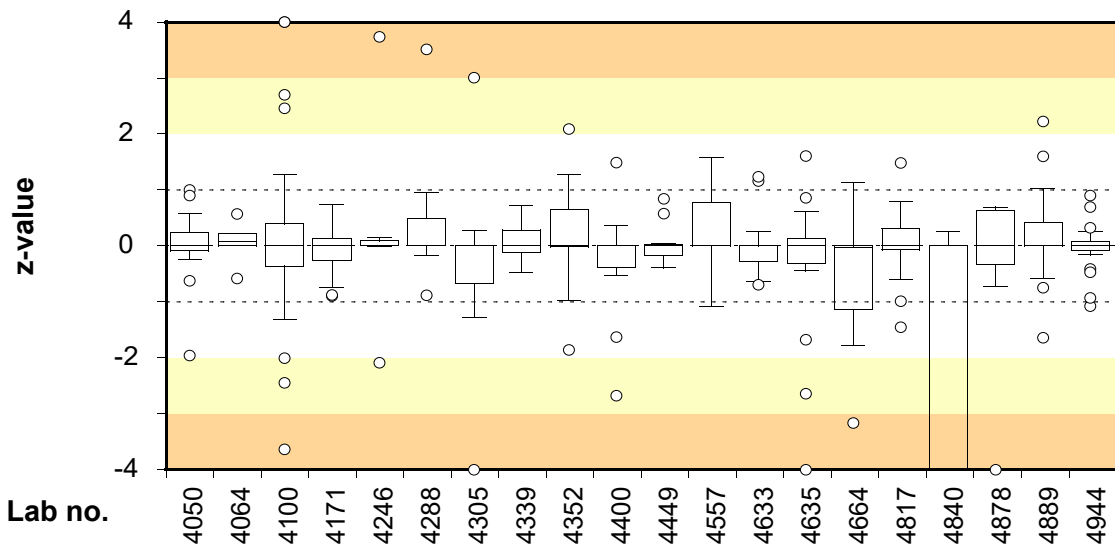
- Z-scores are calculated according to the formula:  $z = (x-m)/s$ , where  $x$  is the result of the individual laboratory,  $m$  is the mean of the results of all participating laboratories, and  $s$  is the standard deviation of the participating laboratories, after removing outliers and false results.
- 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 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  
 $> [highest\ value\ in\ the\ box + 1,5 \times (highest\ value\ in\ the\ box - lowest\ value\ in\ the\ box)]$ .

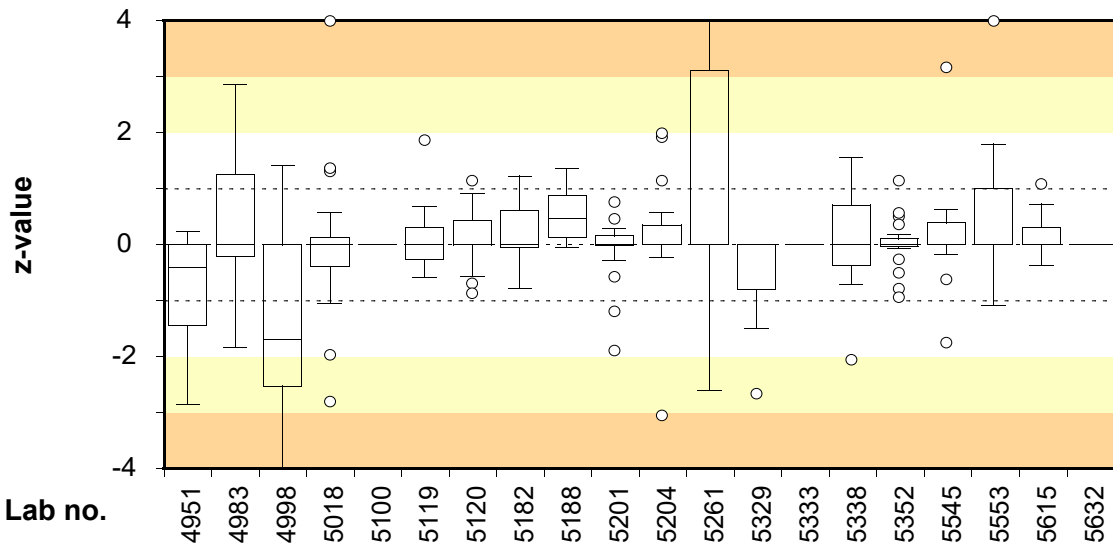




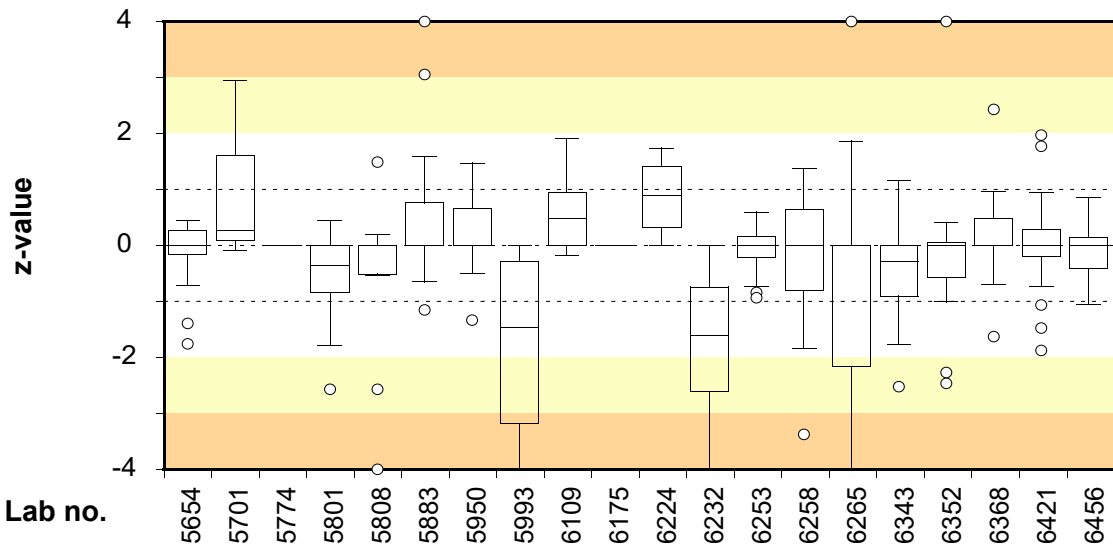
No. of results	25	7	18	11	28	10	13	23	5	34	27	25	14	16	23	18	14	25	20	20
False positive	1	-	-	1	1	2	2	1	1	1	-	2	1	2	-	-	1	5	1	1
False negative	1	2	-	-	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	1	-	1	-	1	-	-	1	-	1
High outliers	-	-	-	-	1	1	-	-	-	-	-	1	-	2	1	-	-	-	-	-



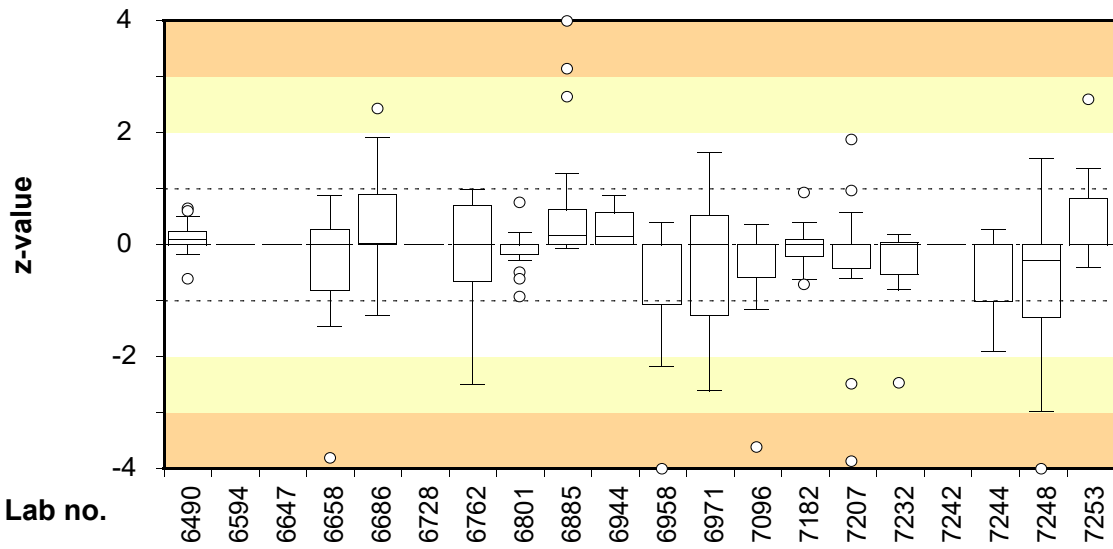
No. of results	18	6	37	23	11	27	16	33	33	16	12	14	16	16	22	20	6	14	24	24
False positive	-	-	1	1	1	-	1	3	3	1	-	1	-	2	2	-	4	1	-	-
False negative	-	-	1	-	-	-	1	-	-	1	-	-	2	1	-	1	2	-	-	-
Low outliers	-	-	-	-	-	-	1	-	-	-	-	-	-	1	1	-	2	2	-	-
High outliers	-	-	2	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-



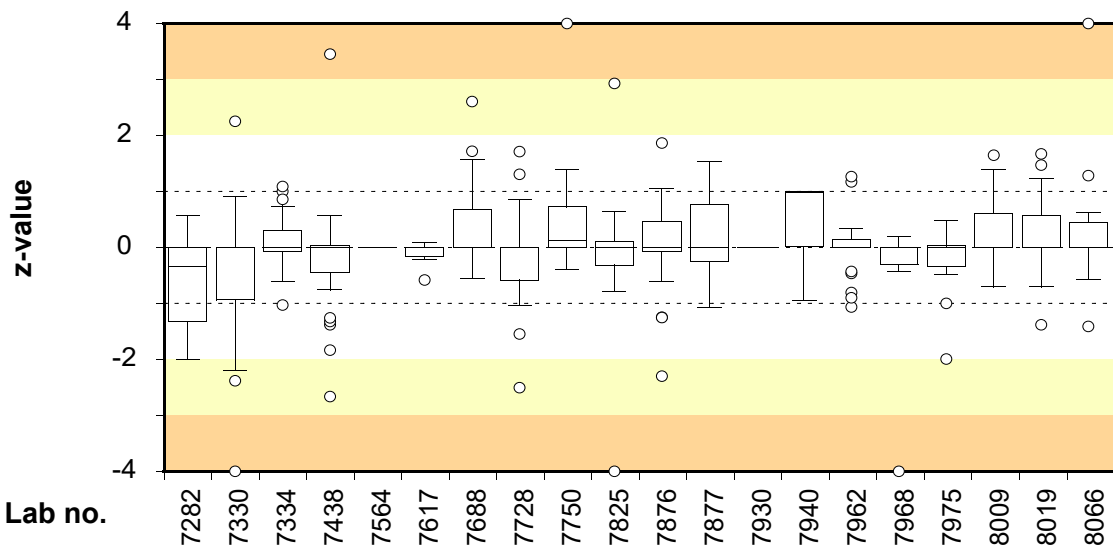
Lab no.	4951	4983	4998	5018	5100	5119	5120	5182	5188	5201	5204	5261	5329	5333	5338	5352	5545	5553	5615	5632
No. of results	14	14	8	34	-	11	33	16	8	18	31	15	22	-	11	23	14	18	25	-
False positive	1	1	1	2	-	1	2	1	-	2	2	-	-	-	1	-	1	-	1	-
False negative	-	-	-	-	-	-	1	1	-	1	-	-	2	-	-	1	-	-	1	-
Low outliers	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	1	-	-	-	-	-	-	-	2	-	-	-	-	1	1	-	-



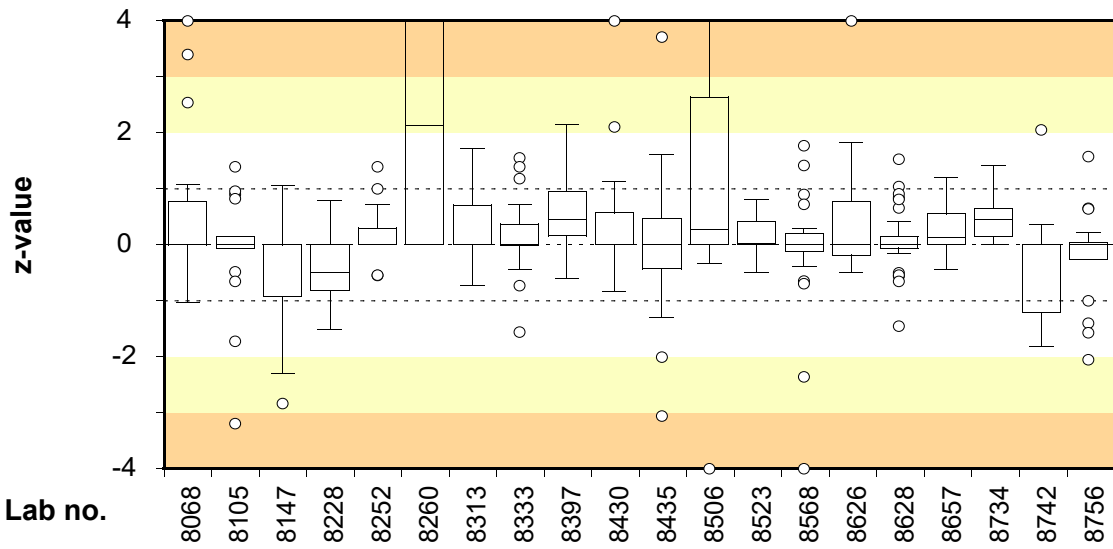
Lab no.	5654	5701	5774	5801	5808	5883	5950	5993	6109	6175	6224	6232	6253	6258	6265	6343	6352	6368	6421	6456
No. of results	15	3	-	14	13	24	39	4	21	-	8	5	18	11	30	27	25	33	32	21
False positive	-	-	-	1	-	-	-	1	-	-	1	1	-	-	-	3	2	-	1	-
False negative	-	-	-	-	2	-	-	1	-	-	-	-	-	1	3	-	-	-	-	-
Low outliers	-	-	-	-	1	-	-	1	-	-	-	1	-	-	2	-	-	-	-	-
High outliers	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	1	-	-	-



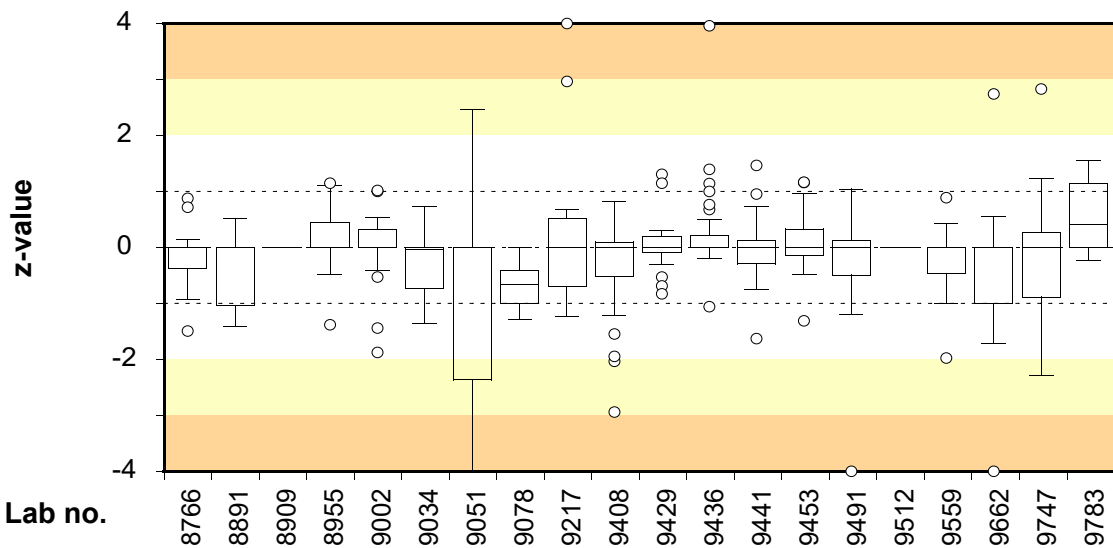
No. of results	21	-	-	13	28	-	8	15	23	12	15	8	9	15	18	9	-	11	36	27
False positive	-	-	-	2	1	-	1	-	1	-	-	1	-	3	-	-	-	-	1	-
False negative	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-
Low outliers	-	-	-	1	-	-	-	-	-	1	-	1	-	1	-	-	-	-	1	-
High outliers	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-



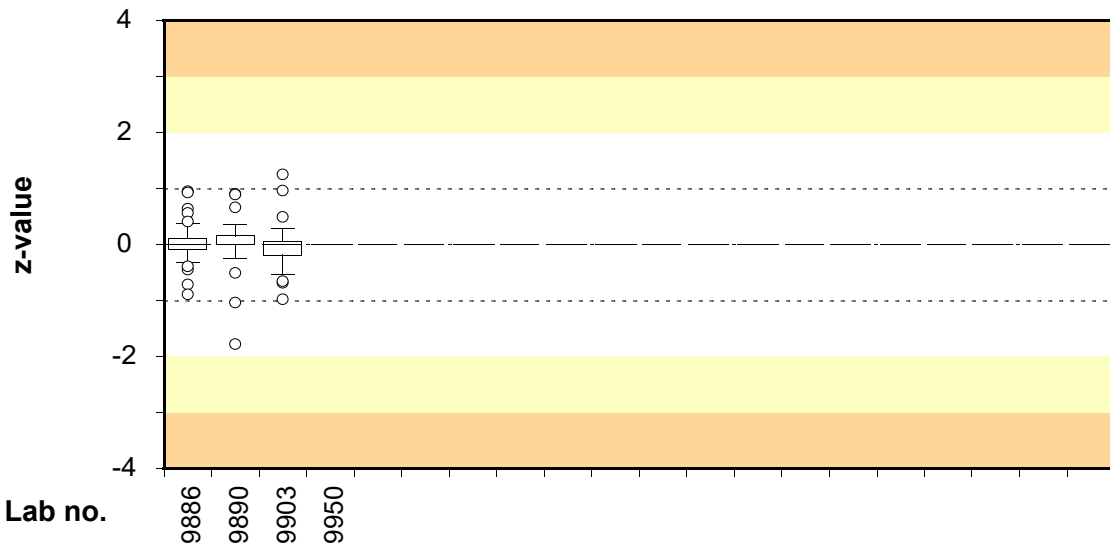
No. of results	21	21	20	27	-	9	31	26	12	17	24	14	-	3	21	7	15	19	34	15
False positive	-	-	-	-	-	-	1	-	-	4	-	1	-	-	-	2	-	2	2	3
False negative	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	1	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-
High outliers	-	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	1



Lab no.	8068	8105	8147	8228	8252	8260	8313	8333	8397	8430	8435	8506	8523	8568	8626	8628	8657	8734	8742	8756
No. of results	24	18	25	15	21	27	24	23	21	17	24	17	20	23	12	35	12	7	9	20
False positive	-	-	1	1	-	-	-	1	1	1	-	-	1	1	-	1	-	2	-	-
False negative	-	-	4	2	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
Low outliers	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-
High outliers	2	-	-	-	-	12	-	-	-	1	1	4	-	-	1	-	-	-	-	-



Lab no.	8766	8891	8909	8955	9002	9034	9051	9078	9217	9408	9429	9436	9441	9453	9491	9512	9559	9662	9747	9783
No. of results	24	21	-	35	28	12	12	5	12	35	21	31	32	18	21	-	21	36	13	9
False positive	-	-	-	1	1	-	-	1	4	1	-	1	1	-	2	-	2	-	1	-
False negative	-	-	-	-	1	-	-	-	2	-	-	1	-	-	-	-	1	-	1	-
Low outliers	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	1	-	-
High outliers	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-



No. of results	31	21	24	-
False positive	1	3	-	-
False negative	1	-	-	-
Low outliers	-	-	-	-
High outliers	-	-	-	-

## Test material and quality control

### Test material

Each laboratory received three sample mixtures with freeze-dried microorganisms, designated A-C. The test material was freeze-dried in portions of 0.5 ml in vials, as described by Peterz and Steneryd (3). Before analysing the samples, the contents of each vial should be dissolved in 254 ml of sterile diluent. The organisms present in the mixtures are listed in Table 2.

**Table 2.** *Microorganisms in the samples*

Sample <sup>1</sup>	Microorganism	Strain	
		SLV no. <sup>2</sup>	Reference <sup>3</sup>
A	<i>Aeromonas hydrophila</i>	SLV-454	CCUG 30 208
	<i>Clostridium perfringens</i>	SLV-442	CCUG 43593
	<i>Staphylococcus warneri</i>	SLV-565	CCUG 61870
	<i>Staphylococcus aureus</i>	SLV-350	CCUG 45099
	<i>Shewanella putrefaciens</i>	SLV-520	CCUG 46538
B	<i>Aspergillus flavus</i>	SLV-480	CBS 282.95
	<i>Bacillus cereus</i>	SLV-518	CCUG 44741
	<i>Brochothrix thermosphacta</i>	SLV-220	CCUG 45641
	<i>Clostridium perfringens</i>	SLV-442	CCUG 43593
	<i>Hanseniaspora uvarum</i>	SLV-555	-
	<i>Shewanella putrefaciens</i>	SLV-520	CCUG 46538
C	<i>Bacillus cereus</i>	SLV-160	CCUG 45098
	<i>Cladosporium cladosporioides</i>	SLV-488	CBS 812.96
	<i>Escherichia coli</i>	SLV-524	CCUG 47554
	<i>Kluyveromyces marxianus</i>	SLV-439	CBS G99-106
	<i>Lactobacillus plantarum</i>	SLV-475	CCUG 30503
	<i>Staphylococcus xylosus</i>	SLV-283	Cheese, 1989

<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 Swedish Food Agency

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



### Quality control of the samples mixtures

In order to allow comparison of all freeze-dried samples, it is essential to have aliquots of homogeneous sample mixtures and equal volume in all vials. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the samples or on 5 vials if an “old” sample mixture was used and the last quality control was performed more than 6 months ago. Homogeneity of a sample mixture is approved if, for each analysis, the values obtained for the test of reproducibility (T) and the test “Index of dispersion” between vials ( $I_2$ ) do not simultaneously exceed 2.6 and 2.0, respectively. (For definitions of T and  $I_2$ , see references 4 and 5 respectively.)

**Table 3.** Concentration mean ( $m$ ),  $I_2$  and T values from the quality control of the sample mixtures;  $m$  is expressed in  $\log_{10}$  cfu (colony forming units) per ml of sample.

Analysis and method	A <sup>1</sup>			B <sup>1</sup>			C <sup>2</sup>		
	m	$I_2$	T	m	$I_2$	T	m	$I_2$	T
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.82	0.46	1.18	4.20	0.19	1.24	4.57	0.82	1.36
Psychrotrophic microorganisms NMKL method no. 86:2013	3.06	1.23	<b>2.61</b>	4.74	0.35	1.17	4.34	1.83	1.81
Enterobacteriaceae NMKL method no. 144:2005	<i>2.69</i> <sup>3</sup>	<i>0.90</i> <sup>3</sup>	<i>2.47</i> <sup>3</sup>	-	-	-	3.65	0.82	1.31
<i>Escherichia coli</i> NMKL method no. 125:2005	-	-	-	-	-	-	3.74	1.53	1.37
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	<i>3.18</i> <sup>3</sup>	<i>0.67</i> <sup>3</sup>	<i>3.15</i> <sup>3</sup>	4.06	0.55	1.51	4.59	0.59	1.28
Coagulase-positive staphylococci NMKL method no. 66:2009	4.21	0.58	1.18	-	-	-	3.25	1.69	<b>4.03</b>
Lactic acid bacteria NMKL method no. 140:2007	-	-	-	-	-	-	3.65	1.07	1.36
<i>Clostridium perfringens</i> NMKL method no. 95:2009	3.11	0.85	1.25	2.56	0.57	1.29	-	-	-
Anaerobic sulphite-reducing bacteria NMKL method no. 56:2015	3.23	1.49	1.32	2.77	1.88	1.43	-	-	-
Aerobic microorganisms in fish products NMKL method no. 184:2006	4.54	<b>3.15</b>	1.30	4.60	<b>2.88</b>	1.77	4.64	1.49	1.43
H <sub>2</sub> S-producing bacteria in fish products NMKL method no. 184:2006	4.12	<b>3.25</b>	1.59	3.55	1.12	<b>4.11</b>	-	-	-
Yeasts NMKL method no. 98:2005	-	-	-	2.38	0.34	1.26	2.49	1.95	1.63
Moulds NMKL method no. 98:2005	-	-	-	2.33	1.04	1.54	2.18	0.13	1.21

- 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> No target organism for the analysis

## References

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### Annex 1 Results of the participating laboratories - April 2020

All results are in log<sub>10</sub> cfu per ml sample. Results reported as "< value" have been regarded as zero. Results reported as "> value" are excluded from the calculations. A dash indicates the analysis was not performed. Outliers and false results are highlighted and summarized for each analysis at the end of the table

Lab no	Vial	Aerobic microorganisms 30 °C			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 <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				
1149	2 1 3	4.71	4.58	4.6	-	-	-	2.49	0	3.85	0	0	3.64	2.85	4.67	4.85	4.28	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.34	2.46	0	2.4	1.3	1149
1237	2 1 3	4.76	4.89	4.08	-	-	-	<1	1.46	3.6	<1	<1	3.54	1.3	3.73	<1	<1	<1	<1	2.23	3.3	2.9	2.48	<1	3.04	2.32	<1	3.34	4	4	3.11	<1	<1	<1	2.15	2.2	<1	1.6	1.48	1237	
1290	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1290	
1545	2 1 3	4.89	4.3	4.58	-	-	-	<0	<0	3.78	<0	<0	3.63	<1	4.32	4.51	3.78	<0	<0	<0	1.78	3.58	3.23	2.41	<0	3.23	2.71	<0	-	-	-	-	<0	2.4	2.34	<0	2.23	1.9	1545		
1594	1 3 2	4.78	4.27	4.63	-	-	-	0	0	3.78	0	0	3.67	0	4.48	4.57	4.02	4.57	0	-	-	-	-	-	3.15	2.66	0	-	-	-	3.6	2.36	0	0	2.42	2.4	0	2.29	2.2	1594	
1970	2 1 3	4.79	4.13	4.14	<1	4.95	3.4	<1	<1	3.49	<1	<1	3.65	<1	4.36	<1	4.58	<1	<1	<1	<1	3.48	3.23	2.73	<1	3.23	2.69	<1	4.43	3.99	4.34	3.68	3.32	<1	<1	2.3	2.18	<1	1.9	1.85	1970
2035	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2035
2064	3 2 1	4.73	3.8	-	-	-	-	1.54	0	-	-	-	-	0	3.99	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.28	-	0	1.73	-	2064
2072	2 3 1	4.82	3.94	4.43	4.62	4.75	4.56	<1	<1	3.56	<1	<1	3.53	<1	4.23	4.36	3.97	<1	<1	<1	<1	3.58	2.63	1.78	<1	2.63	1.78	<1	-	-	-	<1	2.3	2.3	<1	2.4	1.72	2072			
2221	3 1 2	4.87	4.09	4.55	2.79	4.69	4.28	<0	<0	3.65	<0	<0	3.59	<0	3.77	4.5	4.04	<0	<0	4.68	<0	3.63	3.34	2.96	<1	3.04	2.34	<0	-	-	-	<1	2.45	2.28	<1	2.37	1.87	2221			
2317	2 1 3	4.89	3.74	4.26	-	-	-	3.12	<1	3.43	<1	<1	3.58	-	3.58	4.51	3.82	<1	<1	-	-	-	2.57	1.78	<1	2.11	1.97	<1	-	-	-	<1	2.21	2.11	<1	1.86	1.83	2317			
2324	1 3 2	4.84	4.55	4.17	-	-	-	<1	<1	3.61	<1	<1	3.69	<1	4.3	4.55	4.52	<1	<1	-	-	-	-	-	>1	>1	<1	-	-	-	<1	2.25	2.34	<1	2.05	1.97	2324				
2344	2 1 3	4.81	4.3	4.46	-	-	-	0	0	3.69	0	0	3.68	0	4.39	-	4.17	0	0	0	-	-	2.93	2.43	-	2.93	2.44	-	-	-	-	0	2.19	-	0	1.86	-	2344			
2386	2 3 1	4.78	4.34	4.53	-	-	-	<1	<1	3.28	<1	<1	3.7	<1	4.32	3.46	-	-	-	<1	<1	3.74	-	-	-	3.04	2.68	<1	4.13	5.4	4.29	3.23	<1	<1	-	-	-	-	-	2386	
2402	2 3 1	4.89	4.13	4.64	-	-	-	2.57	0	3.64	0	0	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.78	1.38	2.69	1.48	0.85	0.77	2402		
2459	3 1 2	5.92	5.21	5.01	-	-	-	0	0	4.65	0	0	4.66	-	4.87	0	5	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	3.29	4.41	0	3.08	0	2459	
2599	2 3 1	4.94	4.68	4.65	3.15	3.56	3.3	2.2	0	3.69	0	0	3.6	0	3.95	4.51	4.04	0	0	-	-	-	3	2.46	0	-	-	-	4.26	4.42	3.6	4.04	2.48	0	-	-	-	-	-	2599	
2637	3 1 2	4.81	4.15	4.72	-	-	-	2.04	<1	3.66	<1	<1	3.91	<1	4.04	3.91	4.08	<1	<1	<1	<1	3.57	3.34	2.83	<1	3.4	2.85	<1	-	-	-	<1	2.24	2.28	<1	1.95	1.64	2637			
2704	2 3 1	4.83	4.94	4.64	-	-	-	<1	<1	3.61	<1	<1	3.59	<1	4.23	4.58	4.32	<1	<1	-	-	-	-	-	3.26	2.49	<1	3.53	3.96	4.27	-	-	-	-	-	-	-	-	-	2704	
2720	2 1 3	4.78	3.99	4.19	-	-	-	<1	<1	3.57	-	-	-	<1	3.97	4.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.2	2.15	<1	1.77	1.3	2720		
2745	1 3 2	4.68	3.93	4.52	-	-	-	<0	<0	3.81	<0	<0	<0	<1	4.64	4.48	4.43	<0	<0	4.63	<0	3.61	3.23	2.87	<0	-	-	-	-	-	-	<0	2.32	2.41	<0	2.2	2.08	2745			
2794	1 3 2	4.79	3.83	4.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	2.48	0	2.88	0	2794			
2915	1 3 2	4.87	4.32	4.53	-	-	-	<1	<1	3.89	<1	<1	3.43	<2	4.11	4.26	3.87	<1	<1	-	-	-	-	-	-	-	-	-	3.4	4.38	4.28	-	-	-	-	-	-	-	-	2915	
2920	1 3 2	4.89	3.98	4.53	-	-	-	2.49	0	3.62	0	0	3.57	-	-	-	-	-	-	-	-	-	-	-	-	2.76	2.41	0	-	-	-	-	-	-	-	-	-	-	-	-	2920
2941	2 1 3	4.59	3.89	4.21	0	4.8	3.82	0	0	3.46	0	0	3.6	0	3.9	4.46	4.62	0	0	1.09	0	3.57	-	-	-	2.67	2.72	0	-	-	-	0	2.25	2.3	0	1.69	1.17	2941			
3031	3 2 1	-	-	-	-	-	-	-	-	-	0	0	4.08	-	-	-	4.96	1.84	3.38	-	-	-	-	-	-	-	-	4.57	4.08	4.56	3.86	3.62	0	-	-	-	-	-	-	-	3031
3055	3 2 1	4.73	4.06	4.05	-	-	-	2.11	0	3.5	-	-	-	3.1	4.23	4.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1.93	1.88	0	2.15	1.38	0	2.15	1.38	3055
3155	2 3 1	4.76	4.94	4.6	-	-	-	2.2	0	3.78	0	0	3.48	0	4.33	4.26	4.01	0	0	-	-	-	3.11	2.79	0	-	-	-	-	-	-	0	2.24	2.04	0	2.2	2.34	0	2.2	2.34	3155
3243	1 3 2	4.73	3.8	4.17	-	-	-	2.11	0	3.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3243
3305	2 1 3	4.66	3.78	4.36	-	-	-	<1	<1	3.79	<1	<1	3.79	<1	4.04	4.52	3.9	<1	<1	<1	4.32	3.63	<1	2.46	<1	3.23	2.76	<1	4.69	4.59	4.59	3.36	2.65	<1	<1	2.41	2.3	<1	2.28	1.58	3305
3415	3 2 1	4.72	4.32	4.08	-	-	-	<1	<1	3.57	<1	<1	3.6	<1	4.32	4.52	3.9	<1	<1	-	-	-	2.11	1.43	<1	3.11	2.4	<1	-	-	-	<1	2.2	2.18	<1	2.08	1.66	2.08	1.66	3415	
3457	3 1 2	-	-	-	-	-	-	0	0	3.08	0	0	3.75	-	-	-	4.61	0	2.85	1.11	0	3.54	2.83	2.41	0	-	-	-	4.32	4.57	4.42	3.78	2.97	0	0	2.44	2.1	0	2.17	1.49	3457
3515	3 2 1	4.73	4.36	4.86	-	-	-	0.92	0	3.65	0	0	3.86	0	4.14	4.07	3.08	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3515
3543	2 3 1	5.04	3.91	4.65	-	-	-	1.42	<1	3.63	-	-	-	<1	3.78	4.2	4.84	<1	2.93	-	-	-	-	-	-	-	-	-	-	-	<1	3.32	2.78	-	-	-	-	-	-	-	3543
3587	3 2 1	4.75	3.85	4.14	-	-	-	<1	<1	3.61	<1	<1	3.63	<1	3.83	3.19	4.73	<1	<1	-	-	-	-	-	-	3.08	2.6	<1	-	-	-	<1	2.28	2.26	<1	1.85	<1	-	-	-	3587
3626	1 2 3	4.8	3.9	4.7	-	-	-	<1	<1	3.7	<1	<1	3.7	<1	4.2	4.5	4.1	<1	<1	-	-	-	3.1	2.6	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3626
3831	2 3 1	4.87	4.23	4.43	-	-	-	-	-	-	0	0	3.84	-	-	-	-	-	-	0	1.93	3.48	-	-	-	-	-	-	-	-	-	0	2.46	2.55	0	2.18	1.92	0	2.18	1.92	3831
3897	3 1 2	4.4	4	3.47	4.68	4.36	4.07	1.3	2.77	2.73	<1	<1	3.34	<1,30	4.3	4.04	3.84	<1,30	2.6	<1	2.04	3.55	2.25	2.58	<1	-	-	-	-	-	-	<1,30	2.3	2.17	3.11	2.69	2.3	2.69	2.3	3897	
3965	1 3 2	4.78	3.99	4.57	-	-	-	2.58	<1	3.83	<1	<1	3.31	<1	3.89	4.51	4.36	<1	<1	-	-	-	-	-	-	4.29	4.99	4.52	3.88	3.04	<1	-	-	-	-	-	-	-	-	-	3965
4047	1 3 2	4.89	4.47	4.65	-	-	-	1.93	<1	3.67	<1	<1	3.77	<1	3.98	4.44	4.01	<1	<1	-	-	-	-	-	-	-	-	-	-	-	<1	1.6	1.56	<1	2.2	1.7	2.2				

Lab no	Vial	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 <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				
4449	3 2 1	4.74	4.04	4.36	-	-	-	0	0	3.74	0	-	-	0	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	2.26	0	-	2	4449				
4557	2 1 3	4.8	4.89	4.57	-	-	-	3.15	0	3.44	0	0	3.39	-	-	-	4.1	0	0	-	-	-	-	-	-	4.79	4.94	4.55	-	-	-	-	-	-	-	-	4557				
4633	3 2 1	-	-	-	-	-	-	-	-	-	<1	<1	3.81	<1	3.86	4.26	<2	<2	<1	-	-	-	-	3.34	<1	<1	-	-	-	<1	2.18	2.19	<1	1.94	1.91	4633					
4635	2 3 1	3.1	3.49	3.72	-	-	-	1.7	<1	3.51	-	-	-	<1	4.08	4.46	<1	1.9	<1	-	-	-	-	>1	>1	<1	-	-	<1	2.33	2.5	<1	2.08	2.08	4635						
4664	2 3 1	4.82	4.7	4.61	-	-	-	1.94	0	3.64	-	-	-	-	-	-	4.08	0	3	-	-	-	-	2.95	2.18	0	4	4.49	4.18	3	1.49	0	0	2.04	2.06	0	2.13	1.56	4664		
4817	2 1 3	4.66	4.15	4.24	-	-	-	-	-	-	<1	<1	3.32	<1	4.56	<1	4.1	<1	<1	-	-	-	3.08	2.79	<1	-	-	-	<1	2.39	2.23	<1	2.25	1.98	4817						
4840	1 2 3	-	-	-	-	-	-	3.16	<1	2.7	3.16	<1	<1	-	-	-	2.51	<1	4.76	-	-	-	<1	2.61	3.33	-	-	-	-	-	-	-	-	-	-	-	4840				
4878	3 2 1	4.74	3.9	4.56	-	-	-	2.41	0	3.71	-	-	0	1.97	2.48	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.42	2.35	0	2.25	1.9	4878						
4889	1 2 3	4.83	4.04	4.2	-	-	-	0	0	3.57	0	0	3.79	0	4.81	4.45	4.38	0	0	-	-	-	-	2.95	2.04	0	4.42	4.63	4.42	3.53	3	0	0	2.46	2.11	<0	2	1.82	4889		
4944	3 1 2	4.82	4.26	4.46	-	-	-	<0	<0	3.62	<0	<0	3.39	<0	4.3	4.25	4.08	<0	<0	-	-	-	3.05	2.39	<0	-	-	-	<0	2.46	2.11	<0	2	1.82	4944						
4951	2 1 3	4.68	3.59	4.4	-	-	-	2.06	<1	3.08	<1	<1	3.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.33	1.99	<1	1.86	1.47	4951							
4983	1 2 3	4.73	4.54	4.72	-	-	-	1.48	0	3.58	-	-	-	0	3.88	3.8	-	-	-	-	-	-	-	-	-	-	-	0	2.85	2.46	0	2.7	1.78	4983							
4998	3 2 1	4.44	4.82	3.82	-	-	-	2.71	<1	3.25	-	-	-	<1	3.58	3.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4998				
5018	2 3 1	4.69	3.76	4.22	-	-	-	<1	<1	3.09	<1	<1	3.57	<1	3.83	4.4	4.1	<1	<1	1.32	0.6	3.76	3.36	2.57	<1	2.99	2.54	<1	4.76	4.45	4.4	3.36	2.66	<1	<1	1.9	3.32	<1	2.18	2	5018
5100	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5100			
5119	2 3 1	4.71	4.13	4.57	-	-	-	1.83	<1	3.53	<1	<1	3.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.65	2.18	-	-	-	-	-	-	5119				
5120	2 1 3	4.82	3.97	4.54	-	-	-	<1	<1	3.68	<1	<1	3.67	<1	4	4.34	4.26	<1	<1	1.63	5.11	<1	3.32	2.68	<1	3.2	2.51	<1	4.18	4.66	4.53	3.41	3.06	<1	<1	2.15	2.12	<1	2.05	1.96	5120
5182	3 2 1	4.93	4.17	4.62	-	-	-	<1	2.21	3.45	<1	<1	3.45	<1	4.31	<1	-	-	-	-	-	-	-	-	-	-	-	<1	2.29	2.21	<1	2.26	2.17	5182							
5188	2 3 1	-	-	-	3.11	4.79	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.76	4.89	4.54	3.68	-	<2	-	-	-	-	-	-	5188			
5201	2 1 3	4.8	3.7	4.39	-	-	-	3.39	<	<	2.84	<	3.68	<	3.88	4.5	3.76	<	<	<2	2.6	3.66	3.18	2.66	<1	3.03	2.58	<1	<	2.23	2.3	<	2.17	1.89	5201						
5204	3 2 1	4.79	5.04	4.36	3.23	4.69	4.18	2.86	<1	3.6	<1	<1	3.59	<1	3.06	4.36	4.45	<1	<1	<2	2.6	3.66	3.18	2.66	<1	3.03	2.58	<1	<1	2.39	2.43	<1	2.26	1.79	5204						
5261	2 3 1	4.7	4.26	3.73	-	-	-	<1	<1	4.23	-	-	-	<1	4.24	3.86	-	-	-	-	-	-	-	-	-	-	-	<1	3.08	3.02	<1	2.96	2.72	5261							
5329	2 1 3	4.76	3.57	4.19	-	-	-	<1	<1	3.46	<1	<1	3.46	<1	3.81	<1	4.1	<1	<1	<1	<1	3.53	-	-	-	-	-	<1	1.76	2.13	<1	1.85	<1	5329							
5333	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5333				
5338	3 2 1	4.53	3.91	4.52	-	-	-	2.63	0	3.87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.27	2.15	0	2.42	2.1	5338							
5352	2 3 1	4.72	4.1	4.44	-	-	-	0	0	3.45	0	0	3.58	0	4.08	4.43	4.3	0	0	2.81	0	0	-	-	-	-	0	2.27	2.31	0	2.28	1.89	5352								
5545	1 3 2	-	-	-	-	-	-	1.95	0	3.56	-	-	-	0	4.28	4.4	4.66	0	0	-	-	-	-	-	-	-	-	0	2.3	2.35	0	1.96	1.34	5545							
5553	3 1 2	4.85	4.24	4.63	-	-	-	<1	<1	3.48	<1	<1	3.39	<1	4.4	4.66	4.85	<1	<1	-	-	-	3.35	3.12	<1	-	-	-	-	-	-	-	-	-	-	-	5553				
5615	2 3 1	4.83	4.68	4.58	-	-	-	<0	<0	3.68	<0	<0	3.57	<1	4	4.2	4.15	<0	2.92	-	-	-	3.04	2.53	<0	3	2.7	<0	<0	2.26	2.36	<0	2.08	<0	5615						
5632	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5632				
5654	2 1 3	4.81	3.61	4.51	-	-	-	0	0	3.65	-	-	-	<1	4.12	4.38	-	-	-	-	-	-	-	-	-	-	-	0	1.94	2.3	0	2.04	1.63	5654							
5701	2 1 3	5.14	4.17	4.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5701					
5774	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5774				
5801	2 3 1	4.57	3.85	4.3	-	-	-	2.3	<1	3.67	-	-	-	<1	3.48	3.6	-	-	-	-	-	-	-	-	-	-	-	<1	2.34	2.2	<1	2	1.7	5801							
5808	1 2 3	3.8	4.85	4.32	-	-	-	-	-	-	0	0	3.11	0	3.9	0	-	-	-	-	-	-	-	-	-	-	-	0	2.26	2.17	0	2.18	0	5808							
5883	3 2 1	4.79	3.93	4.1	-	-	-	0	0	3.51	0	0	3.76	<1	3.89	4.46	4.01	0	0	-	-	-	3.19	3.05	0	-	-	0	3.3	2.45	0	2.95	2.23	5883							
5950	2 3 1	4.72	4	4.31	2.84	4.57	4.19	<1	<1	3.54	<1	<1	3.6	<1	4.03	4.58	4.2	<1	<1	<1	<1	3.69	3.15	2.67	<1	3.23	2.73	<1	4.6	4.57	4.16	3.85	3.32	<1	<1	2.45	2.48	<1	2.04	2.07	5950
5993	1 3 2	4.71	0	3.79	-	-	-	0.85	0	2.54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5993			
6109	2 3 1	4.88	4.67	4.66	-	-	-	-	-	-	0	0	3.56	<1	4.32	4.49	-	-	-	<1	<1	3.66	-	-	-	3.18	2.81	0	-	-	-	0	2.41	2.4	0	2.64	2.18	6109			
6175	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6175			
6224	3 2 1	4.94	4.86	4.68	-	-	-	2.56	<1	3.9	-	-	-	<2	4.29	4.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6224			
6232	1 3 2	4.46	3.52	4.2	-	-	-	1.6	0	2.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6232			
6253	2 1 3	4.8	3.85	4.34	-	-	-	-	-	-	-	-	-	0	3.76	4.38	4.08	0	0	-	-	-	-	-	3.18	2.49	0	-	-	-	0	2.4	2.28	0	1.93	1.65	6253				
6258	3 2 1	4.88	4.8	4.63	0	4.87	4.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.16	2.1	0	1.63	0.88	6258							
6265	1 2 3	4.85	3.6	3.2	2.68	4.16	0	0	0	2.87	0	0	3.54	0	0	0	4.02	0	0	-	-	-	2.51	1.78	0	2.51	1.78	0	4.76	3.4	3.9	-	-	0	2.43	3.25	0	2.63	2.15	6265	
6343	1 3 2	4.66	3.81	4.32	-	-	-	2.31	<0	3.61	<0	<0	3.53	<0	3.78	4.2	3.97	3.67	<0	<0	1.7	3.42	3.11	2.38	<0	2.54	2.13	<0	<0	2.51	1										

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 <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	A	B	C	
6728	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6728				
6762	1 2 3	4.9	3.65	3.76	-	-	-	1.55	<1	3.67	<1	<1	3.77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6762				
6801	2 3 1	4.78	4	4.24	-	-	-	-	-	-	0	0	3.42	-	-	-	4.09	0	0	-	-	-	-	-	-	-	-	-	-	-	-	0	2.27	2.37	0	2.05	1.9	6801			
6885	1 2 3	4.82	4.5	4.52	-	-	-	2.42	0	3.64	0	0	3.58	0	4.1	4.63	4.87	0	0	-	-	-	3.2	2.58	0	-	-	-	-	-	0	2.9	2.28	0	2.84	2.2	6885				
6944	1 3 2	-	-	-	-	-	-	-	-	-	0	0	3.73	-	-	-	-	-	0	0	3.66	-	-	-	-	-	-	-	-	-	0	2.34	2.39	0	2.23	2.03	6944				
6958	2 3 1	4.65	3.75	3.84	-	-	-	<1	<1	3.64	-	-	-	<1	3.68	2.71	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.21	2.15	<1	2.21	1.95	6958					
6971	3 2 1	4.8	4.92	4.62	-	-	-	2.53	0	3.4	-	-	-	0	3.59	3.59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6971					
7096	2 1 3	-	-	-	-	-	-	-	-	-	<0,5	<0,5	3.66	-	-	-	-	-	-	-	-	-	-	-	-	3.97	4.52	4.4	3.18	1.3	<1	-	-	-	-	-	7096				
7182	1 3 2	4.74	4.38	4.48	-	-	-	2.86	<1	3.48	<1	<1	3.46	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.27	2.24	<1	2.38	1.89	7182						
7207	1 3 2	5.01	4.63	4.37	-	-	-	<1	<1	2.9	-	-	-	<1	4.26	4.18	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.2	2.16	<1	1.98	1.9	7207						
7232	2 3 1	4.48	4.04	4.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	2.32	2.26	0	1.91	1.87	7232						
7242	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7242					
7244	1 3 2	4.62	3.82	3.91	-	-	-	-	-	-	<0,48	<0,48	3.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.15	2.08	<1	2.2	<1	<1	2.24	7244				
7248	3 2 1	4.79	3.17	4.03	2.3	3.75	3.89	<1	<1	3.36	<1	<1	3.48	<1	4	4.28	3.86	<1	<1	4.48	<1	<1	2.95	2.48	<1	2.91	2.28	<1	3	3.96	4.19	3	1	<1	<1	1.99	2.49	<1	1.89	<1	7248
7253	1 2 3	4.88	4.15	4.68	-	-	-	<1	<1	3.82	<1	<1	3.75	<1	4.93	4.66	4.26	<1	<1	-	-	3.2	2.72	<1	3.25	2.79	<1	-	-	<1	2.28	2.19	<1	2.3	2.07	7253					
7282	1 3 2	4.58	4.46	4.31	-	-	-	0	0	3.33	0	0	3.45	0	3.62	4.04	3.99	0	0	-	-	-	-	-	-	-	-	-	0	1.99	2.16	0	1.59	1.46	7282						
7330	1 3 2	4.49	4.6	4.53	-	-	-	0	0	3.41	0	0	3.18	0	3.76	4.21	3.23	0	0	-	-	-	-	-	-	-	-	-	0	1.95	2.6	0	2	1.85	7330						
7334	1 2 3	4.87	3.95	4.36	-	-	-	<1	<1	>1	<1	<1	>1	<1	4.24	4.31	4.04	<1	<1	-	-	-	-	-	3.29	2.8	<1	-	<1	2.3	2.22	<1	1.85	2.08	7334						
7438	1 2 3	4.72	4.04	4.46	-	-	-	<1	<1	3.63	<1	<1	3.45	<1	4.13	4.45	4.71	<1	<1	-	-	2.59	2.07	<1	2.51	2.13	<1	-	<1	2.3	2.06	<1	2.16	1.8	7438						
7564	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7564				
7617	1 3 2	4.79	3.96	4.35	-	-	-	<1	<1	3.61	<1	<1	3.61	<1	4.06	4.26	4.06	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7617				
7688	3 2 1	4.96	3.97	4.46	<1	4.48	4	1	<1	3.61	<1	<1	3.83	<1	4.15	4.34	4.26	<1	<1	<1	<1	3.9	3.34	2.67	<1	3.15	2.43	<1	-	<1	2.59	2.25	<1	2.59	2.25	7688					
7728	2 1 3	4.94	3.91	4	-	-	-	0	0	3.63	0	0	3.66	0	4	0	4.4	0	0	-	-	-	2.94	2.32	0	3.26	1.8	0	-	0	2.08	2.15	0	2.08	1.67	7728					
7750	3 1 2	4.8	4.04	4.58	-	-	-	<1	<1	3.7	<1	<1	2.57	<1	4.53	4.32	4.62	2.36	2.93	<1	<1	1.64	3.59	-	-	-	-	<1	2.22	2.15	<1	2.16	1.61	7750							
7825	3 1 2	4.81	4.03	4.48	-	-	-	3.12	<1	3.7	<1	<1	2.57	<1	4.62	2.36	2.93	<1	<1	1.64	3.59	-	-	-	-	-	-	<1	2.22	2.15	<1	2.16	1.61	7825							
7876	1 3 2	4.5	3.95	4.87	-	-	-	<1	<1	3.74	<1	<1	3.79	<1	4.23	4.32	4.28	<1	<1	-	-	-	3.19	2.67	<1	-	-	<1	2.04	2.23	<1	2.07	1.48	7876							
7877	3 1 2	4.75	4.87	4.68	2.96	4.92	4.37	1.48	<1	3.4	-	-	-	<1	4.43	4.1	-	-	-	-	-	-	-	-	-	-	-	<1	2.15	2.33	-	-	-	-	-	7877					
7930	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7930				
7940	1 2 3	4.9	3.8	4.65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7940				
7962	2 3 1	4.8	4.01	4.48	-	-	-	0	0	3.61	0	0	3.62	0	4.15	4.61	4.32	0	0	-	-	-	-	-	-	-	-	-	0	2.2	2.09	0	1.91	1.58	7962						
7968	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.87	5.11	3.56	1.6	2.59	0	3.08	2.45	0	-	-	-	-	-	-	-	7968					
7975	1 3 2	4.81	4.42	4.41	-	-	-	0	0	3.66	-	-	-	-	3.74	0	0	0	0	-	-	-	-	-	-	-	-	-	0	2.24	2.1	0	2	1.7	7975						
8009	2 3 1	4.74	4.81	4.71	-	-	-	-	-	-	0	0	3.7	-	3.97	0	0	0	0	1.15	0.78	3.58	-	-	-	-	-	4.39	4.83	4.71	-	0	2.35	2.3	0	2.3	1.78	8009			
8019	3 1 2	4.96	4.69	4.54	-	-	-	2.41	0	3.62	0	0	3.77	<1	4.02	3.92	3.97	0	0	<1	2.49	3.73	3.19	2.62	0	3.15	2.63	0	4.41	4.48	4.47	4	2.57	0	<1	2.44	2.26	<1	2.46	1.78	8019
8066	1 3 2	-	-	-	-	-	-	-	-	-	0	0	3.71	0	3.88	4.2	4.84	0	2.65	4.24	4.55	3.45	-	-	-	4.26	4.69	4.43	3.63	3.37	0	-	-	-	-	-	8066				
8068	1 3 2	-	-	-	-	-	-	-	-	-	0	0	3.71	0	4.91	4.48	4.7	0	0	0	0	3.71	3.3	2.81	0	4.18	2.64	0	-	0	2.16	2.2	0	1.85	1.85	8068					
8105	1 3 2	4.89	4.81	4.64	-	-	-	0	0	3.62	0	0	3.62	0	3.5	3.43	4.11	0	0	-	-	-	-	-	-	-	-	-	0	2.19	2.38	0	1.95	1.82	8105						
8147	1 2 3	4.69	4.03	4.09	-	-	-	0	0	3.18	0	0	3.06	<1	4.01	4.58	3.93	<1	<1	<1	2.36	3.46	0	0	0	0	0	-	0	2.18	2.2	0	1.78	1.3	8147						
8228	3 2 1	4.72	3.76	4.15	3.85	4.34	4.32	1.9	0	3.69	-	-	-	0	3.57	3.91	-	-	-	-	-	-	-	-	-	-	-	-	0	2.16	2.15	0	0	0	0	0	8228				
8252	3 2 1	4.95	4.4	4.65	-	-	-	<1	<1	3.61	<1	<1	3.49	<1	3.89	4.49	4.11	<1	<1	-	-	-	-	-	-	-	-	<1	2.4	2.3	<1	2.18	1.85	8252							
8260	2 3 1	7.96	6.27	6.53	-	-	-	0	0	4.81	0	0	4.79	0	5.38	5.68	5.24	0	0	-	-	4.44	2.8	0	4.4	4.09	0	-	0	2.7	2.59	0	3.58	2.04	8260						
8313	3 1 2	4.93	4.93	4.71	-	-	-	0	0	3.46	0	0	3.65	<1	4.64	4.53	4.17	0	0	-	-	-	-	-	3.28	2.61	0	-	0	2.39	2.17	0	1.98	1.79	8313						
8333	2 3 1	4.97	4.02	4.75	-	-	-	1.24	0	3.46	0	0	3.3	<1	4.06	4.39	-	-	-	-	<1	<1	3.74	-	-	3.09	2.7	0	-	0	2.24	2.21	0	2.23	2.02	8333					
8397	3 2 1	4.85	4.83	4.64	-	-	-	-	-	3.67	-	-	3.79	-	3.87	4.38	4.48	-	-	-	3.71	3.72	2.91	2.64	-	3.17	2.68	-	4.23	4.58	4.49	3.42	3.16	-	2.3	2.5	-	2.17	-	8397	
8430	2 3 1	5.04	4.7	4.54	-	-	-	2.42	<1	4.79	<1	<1	3.7	-	4	<1	<1	-	-	-	-	-	-	-	-	-	-	<1	2.3	2.14	<1	1.9	1.84	8430							
8435	2 1 3	4.94	4.08	4.46	-	-	-	<1	<1	3.49	<1	<1	3.69	&lt																											

Lab no	Vial	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 <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				
8766	1 2 3	4.76	4.01	4.43	-	-	-	0	0	3.59	0	0	3.6	0	4.36	3.89	3.99	0	0	-	-	-	2.97	2.32	0	-	-	-	-	-	-	0	2.11	2.11	0	2.32	1.8	8766			
8891	3 2 1	4.72	3.72	4.07	-	-	-	<1	<1	3.53	<1	<1	3.69	<1	3.6	4.38	3.91	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	<1	2.05	2.14	<1	1.83	1.54	8891			
8909	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8909				
8955	1 3 2	4.74	4.08	4.64	-	-	-	2.42	<1	3.51	<1	<1	3.56	<1	4.08	4.59	4.18	<1	<1	-	-	-	3.32	2.72	<1	4.51	4.49	4.48	3.81	2.67	<1	<1	2.34	2.04	<1	2.15	1.98	8955			
9002	1 2 3	4.81	4.03	4.53	1.48	4.77	0	0.88	<0,7	3.54	<0,70	<0,7	3.78	<0,70	4.04	4.29	4.19	0	0	<0,70	<0,7	3.65	-	-	-	2.77	2.35	0	-	-	-	0	2.33	2.41	0	2.26	1.93	9002			
9034	2 3 1	4.77	3.63	4.09	2.79	4.12	4	<1	<1	3.72	<1	<1	3.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9034				
9051	1 3 2	-	-	-	-	-	-	0	0	4.03	0	0	3.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	1.53	1.41	0	1.54	1.11	9051				
9078	3 2 1	4.73	3.66	4.14	-	-	-	1.26	0	3.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9078				
9217	2 1 3	4.63	3.9	4.49	-	-	-	2.03	<1	3.71	-	-	-	2.7	3.85	4.3	3.94	<1	<1	-	-	-	-	-	-	-	-	-	-	-	3.22	<1	3.16	2.6	<1	2.68	9217				
9408	1 2 3	4.42	4.23	4.43	-	-	-	3.14	0	3.63	0	0	3.65	<1	4.12	3.88	4.01	<1	<1	0	0	3.66	2.9	2.72	<1	3.84	4.69	4.18	3.57	2.58	<1	0	2.24	1.94	0	1.6	1.78	9408			
9429	3 2 1	4.94	4.08	4.46	-	-	-	-	-	-	-	-	-	<1	4.04	4.15	4.11	<1	<1	-	-	-	3.11	2.56	<1	3.04	2.57	<1	-	-	-	<1	2.15	2.43	<1	2.18	1.6	9429			
9436	2 1 3	4.79	4.15	4.38	<1	4.53	3.75	<1	<1	3.56	<1	<1	3.72	<1	4.53	4.5	4.8	<1	<1	4.62	<1	3.61	3	2.46	<1	3.32	2.53	<1	-	-	-	<1	2.48	2.3	<1	2.26	1.84	9436			
9441	1 2 3	-	-	-	1.7	4.41	4.04	-	-	-	0	0	3.49	0	4.2	4.28	4	0	0	0	5.18	3.69	3.11	2.64	0	3.04	2.7	0	4.11	4.23	4.32	3.4	2.68	0	0	2.57	2.4	0	2.18	1.85	9441
9453	1 3 2	4.82	4	4.06	-	-	-	<1	<1	3.51	<1	<1	3.51	<1	4.02	4.3	4.21	<1	<1	-	-	-	-	-	-	-	-	-	-	<1	2.51	2.19	<1	2.44	2.11	<1	2.44	2.11	9453		
9491	3 2 1	-	-	-	-	-	-	<1	<1	3.46	<0,48	<0,48	3.04	<1	4.11	4.16	3.16	1.78	2.88	-	-	-	2.77	2.81	<1	2.99	2.76	<1	4.41	4.89	4.54	3.33	2.32	<1	-	-	-	-	-	9491	
9512	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9512	
9559	3 1 2	4.83	4.07	3.89	-	-	-	2.7	<1	3.64	<1	<1	3.76	4.05	4.21	<1	3.93	<1	<1	-	-	-	2.98	2.22	<1	-	-	-	-	-	-	<1	2.31	2.21	<1	1.86	1.57	9559			
9662	2 3 1	4.61	3.71	4.13	-	-	-	<1	<1	4.08	<1	<1	3.27	<1	3.75	4.29	4.15	<1	<1	<1	<1	3.67	2	2.4	<1	3.15	2.63	<1	3.77	4.39	4.16	3.12	2.42	<1	<1	2.11	2.08	<1	2.24	1.76	9662
9747	1 2 3	4.93	5.43	4.08	-	-	-	3.12	<1	3.66	-	-	-	<1	3.69	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9747	
9783	2 3 1	4.92	4.88	4.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9783	
9886	1 3 2	4.75	4.28	4.56	3.04	5.08	4.3	2.74	<1	3.6	<1	<1	3.46	<1	4.38	<1	4.17	<1	<1	<1	<1	3.65	2.93	2.4	<1	3.08	2.41	<1	-	-	-	<1	2.28	2.22	<1	1.89	1.92	9886			
9890	2 1 3	4.72	4.28	4.49	-	-	-	2.79	0	3.71	0	0	3.62	0	4.11	4.23	3.78	0	2.83	0	2.45	3.71	-	-	-	-	-	-	-	-	-	-	0	2.46	2.3	0	1.85	1.78	9890		
9903	2 3 1	4.74	3.92	4.23	-	-	-	<0	<0	3.56	<0	<0	3.41	<0	4.39	4.24	4	<0	<0	-	-	-	3.11	2.57	<0	-	-	-	-	-	-	<0	2.53	2.33	<0	2.09	1.87	9903			
9950	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9950	

N	154	153	153	23	23	23	135	135	135	119	119	118	120	123	120	110	109	109	54	54	54	60	60	58	68	68	69	38	38	38	33	32	32	131	131	130	127	127	125	N		
Min	3.1	0	3.2	0	3.56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3.4	3.6	3	0	0	0	0	0	0	0	0	0	Min	
Max	7.96	6.27	6.53	4.68	5.91	4.64	3.39	2.77	4.81	3.16	0	4.79	4.05	5.38	5.68	5.24	4.57	4.76	4.68	5.18	4.61	4.44	3.12	3.33	4.40	4.09	0	4.79	5.40	4.76	4.04	3.86	0	3.22	3.32	4.41	3.11	3.58	3.04	Max		
Med	4.79	4.08	4.44	3.08	4.69	4.18	0	0	3.61	0	0	3.62	0	4.08	4.34	4.08	0	0	0	0	3.63	3.11	2.59	0	3.12	2.54	0	4.24	4.51	4.43	3.48	2.68	0	0	2.28	2.23	0	2.15	1.85	Med		
m	4.780	4.210	4.394	3.149	4.621	4.108	0	0	3.590	0	0	3.592	0	4.069	4.293	4.095	0	0	0	0	3.608	3.038	2.526	0	3.077	2.496	0	4.203	4.507	4.406	3.513	2.828	0	0	2.284	2.253	0	2.126	1.836	m		
s	0.122	0.430	0.255	0.891	0.477	0.338	0	0	0.179	0	0	0.188	0	0.331	0.270	0.178	0	0	0	0	0.112	0.244	0.330	0	0.213	0.278	0	0.405	0.369	0.184	0.291	0.423	0	0	0.196	0.154	0	0.269	0.284	s		
u <sub>(lg)</sub>	0.010	0.035	0.021	0.210	0.099	0.074	0	0	0.016	0	0	0.018	0	0.030	0.027	0.020	0	0	0	0	0.016	0.034	0.044	0	0.027	0.035	0	0.066	0.060	0.030	0.051	0.083	0	0	0.018	0.014	0	0.025	0.027	u <sub>(lg)</sub>		
F+	0	0	0	0	0	0	63	3	0	2	0	0	6	0	0	0	7	12	15	25	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F+	
F-	0	1	0	5	0	2	0	0	1	0	0	2	0	1	13	3	0	0	0	0	2	3	3	0	1	2	0	0	0	0	0	3	0	0	3	1	0	2	10	0	F-	
<	3	0	1	0	0	0	0	0	7	0	0	1	0	2	4	4	0	0	0	0	0	3	0	0	1	1	0	0	0	1	0	3	0	0	2	2	0	1	1	0	<	
>	2	1	1	0	0	0	0	0	3	0	0	3	0	1	1	22	0	0	0	0	1	1	0	0	2	1	0	0	0	0	0	0	0	0	5	6	0	0	4	1	0	>
< OK	4.40	3.17	3.47	1.48	3.55	3.30	0	0	3.08	0	0	2.91	0	3.05	3.43	3.74	0	0	0	0	3.30	2.25	1.43	0	2.51	1.78	0	3.00	3.40	3.90	3.00	2.27	0	0	1.60	1.88	0	1.54	0.87	< OK		
> OK	5.14	5.43	5.01	4.68	5.91	4.64	0	0	4.24	0	0	4.08	0	4.93	4.85	4.52	0	0	0	0	3.90	3.36	3.12	0	3.40	3.23	0	4.79	5.40	4.76	4.05	3.86	0	0	2.90	2.78	0	2.97	2.73	> OK		

N = number of analyses performed      Max = highest reported result      m = mean value      F+ = false positive      < = low outlier      < OK = lowest accepted value      u<sub>(lg)</sub> = measurement uncertainty for assigned value (m)  
Min = lowest reported result      Med = median value      s = standard deviation      F- = false negative      > = high outlier      > OK = highest accepted value

The analysis is not evaluated  
 Outlier, false positive or false negative  
 Results "larger than" are not evaluated



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-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					
4339	1 3 2	0.326	-0.140	-0.212				0	0.394	0	0	0.307	0	0.124	0.433	-0.085	0	0	0.645	0.457	0.286	0	0.718	-0.347	0	-0.008	-0.344	0.563	-0.115	-0.444	0	0	-0.274	-0.476	0	-0.321	-0.092	4339				
4352	2 1 3	0.654	2.090	1.120				0	0.449	0	0	0.680	0	-0.299	0.914	-0.984	0	0	-1.856	0.580	0.316	0	1.235	0.589	0	-0.305	-0.398	0.997	-0.184	-0.822	0	0	-0.019	0.694	0	-0.321	1.280	4352				
4400	3 2 1	0.080	-0.280	-0.134				0	-0.502	0	0	0.361	0	-1.628	-2.677																	0	-0.530	0	-0.061	1.490	4400					
4449	3 2 1	-0.330	-0.396	-0.134				0	0.841	0	0	0.208																			0	0.044	0	0	0.576	4449						
4557	2 1 3	0.162	1.579	0.689				0	-0.838	0	0	-1.078				0.027	0	0								1.447	1.175	0.780									4557					
4633	3 2 1							0	0	0	1.160	0	-0.631	-0.122								1.235		0						0	-0.530	-0.411	0	-0.692	0.260	4633						
4635	2 3 1	-4.000	-1.674	-2.642				0	-0.446	0	0	0.034	0.618																	0	0.237	1.603	0	-0.173	0.858	4635						
4664	2 3 1	0.326	1.137	0.846				0	0.282							-0.085	0	0							-0.595	-1.138	0	-0.502	-0.046	-1.226	-1.766	-3.162	0	0	-1.245	-1.256	0	0.013	-0.970	4664		
4817	2 1 3	-0.987	-0.140	-0.604				0	0	0	-1.450	0	1.484			0.027	0	0		0.170	0.801	0								0	0.544	-0.151	0	0.459	0.506	4817						
4840	1 2 3							0	-4.000	0						-4.000	0				0.255																	4840				
4878	3 2 1	-0.330	-0.721	0.650				0	0.673	0	0	0	-4.000	-4.000																	0	0.697	0.629	0	0.459	0.225	4878					
4889	1 2 3	0.433	-0.394	-0.745				0	0	0	0.1027	0	2.227	0.570	1.601	0	0							-0.577	-1.639	0	0.522	0.342	0.048	0.061	0.407	0						4889				
4944	3 1 2	0.326	0.115	0.258				0	0	0	0.170	0	-1.078	0	0.698	-0.160	-0.085	0	0	0.048	-0.412	0									0	0.901	-0.931	0	-0.469	-0.056	4944					
4951	2 1 3	-0.822	-1.441	0.023				0	-2.854	0	0	-1.450																			0	0.237	-1.711	0	-0.989	-1.287	4951					
4983	1 2 3	-0.396	0.775	1.261				0	-0.054	0	0		0	-0.586	-1.829															0	2.869	1.357	0	2.125	-0.204	4983						
4998	3 2 1	-2.791	1.416	-2.250				0	-1.902	0	0		0	-1.477	-4.000																								4998			
5018	2 3 1	-0.740	-1.046	-0.682				0	0	0	-2.798	0	0	-0.119	0	-0.722	0.396	0.027	0	0	1.360	1.316	0.134	0	-0.408	0.157	0	1.373	-0.154	-0.033	-0.528	-0.397	0	0	-1.960	4.000	0	0.199	0.576	5018		
5100	3 2 1																																							5100		
5119	2 3 1	-0.576	-0.187	0.689				0	-0.334	0	0	0.627																			0	1.872	-0.476						5119			
5120	2 1 3	0.326	-0.559	0.571				0	0	0	0.505	0	0	0.414	0	-0.208	0.174	0.927	0	0	1.152	0.468	0	0.578	0.049	0	-0.058	0.415	0.672	-0.356	0.549	0	0	-0.683	-0.866	0	-0.284	0.436	5120			
5182	3 2 1	1.228	-0.094	0.885				0	-0.782	0	0	0	-0.758	0	0.728																0	0.033	-0.281	0	0.496	1.174				5182		
5188	2 3 1				-0.044	0.355	0.273																				1.373	1.039	0.726	0.573	0									5188		
5201	2 1 3	0.162	-1.186	-0.016				0	0	0	0.467	0	-0.571	0.766	-1.884	0	0														0	-0.274	0.304	0	0.162	0.190	5201					
5204	3 2 1	0.080	1.927	-0.134	0.091	0.145	0.214	0	0.058	0	0	-0.012	0	-3.048	0.248	1.995	0	0	0	0.467	0.580	0.407	0	-0.220	0.301	0				0	0.544	1.149	0	0.496	-0.162	5204						
5261	2 3 1	-0.655	0.111	-2.606				0	0	0	3.609	0	0	0.513	-1.587																0	4.000	4.000	0	3.108	3.123	5261					
5329	2 1 3	-0.138	-1.492	-0.810				0	0	0	-0.713	0	0	-0.794		0.010	0	0	0	-0.681										0	-2.658	-0.799	0	-1.044				5329				
5333	1 3 2																																							5333		
5338	3 2 1	-2.053	-0.698	0.493				0	1.569																						0	-0.070	-0.671	0	1.089	0.928	5338					
5352	2 3 1	-0.494	-0.257	0.180				0	0	0	-0.782	0	0	-0.065	0	0.034	0.507	1.152	0	0	-0.935	0								0	-0.070	0.369	0	0.570	0.190	5352						
5545	1 3 2							0	-0.166	0	0	0.638	0.396	3.175	0	0															0	0.084	0.629	0	-0.618	-1.744				5545		
5553	3 1 2	0.572	0.069	0.924				0	0	0	-0.614	0	0	1.000	1.359	4.000	0	0		1.275	1.803	0																	5553			
5615	2 3 1	0.408	1.091	0.728				0	0	0	0.505	0	0	-0.119	0	-0.208	-0.345	0.308	0		0.007	0.012	0	-0.361	0.733	0				0	-0.121	0.694	0	-0.173				5615				
5632	2 1 3																																							5632		
5654	2 1 3	0.244	-1.395	0.454				0	0	0	0.338			0	0.154	0.322															0	-1.756	0.304	0	-0.321	-0.718				5654		
5701	2 1 3	2.951	-0.085	0.266																																				5701		
5774	2 3 1																																								5774	
5801	2 3 1	-1.725	-0.837	-0.369				0	0.449				0	-1.779	-2.566																0	0.288	-0.346	0	-0.469	-0.478				5801		
5808	1 2 3	-4.000	1.486	-0.291				0	0	0	-2.569	0	-0.510																		0	-0.121	-0.541	0	0.199					5808		
5883	3 2 1	0.080	-0.651	-1.153				0	0	0	0.893	0	-0.541	0.618	-0.479	0	0														0	4.000	1.279	0	3.057	1.385				5883		
5950	2 3 1	-0.494	-0.489	-0.330	-0.347	-0.106	0.243	0	0	0	0.041	0	-0.118	1.062	0.589	0	0	0	0	0.735	0.457	0.437	0	0.718	0.841	0	0.979	0.171	-1.334	1.158	1.163	0	0	0.850	1.473	0	-0.321	0.823	5950			
5993	1 3 2	-0.576		-2.367				0	-4.000																																5993	
6109	2 3 1	0.818	1.068	1.042				0	0	0	-0.172	0	0.759	0.729						0	0	0.467				0.484	1.128	0			0	0.646	0.954	0	1.906	1.209				6109		
6175	1 2 3																																								6175	
6224	3 2 1	1.310	1.509	1.120				0	1.737				0	0.668	0.655																										6224	
6232	1 3 2	-2.611	-1.608	-0.745				0	-4.000																																	6232
6253	2 1 3	0.162	-0.837	-0.212				0					0	-0.933	0.322	-0.085	0	0							0.484	-0.023	0				0	0.595	0.174	0	-0.729	-0.654				6253		
6258	3 2 1	0.778	1.376	0.918		0.521	0.219																									0	-0.625	-0.982	0	-1.849	-3.378				6258	
6265	1 2 3	0.572	-1.418	-4.000	-0.527	-0																																				





Lab no.	Vial	Aerobic microorganisms 30 °C			Psychrotrophic microorganisms			Entero-bacteriaceae			<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				
8734	3 1 2	0.490	1.416	0.454																																	8734				
8742	3 1 2	-1.315	-1.209	-1.819							0 0	0.361																									8742				
8756	3 1 2	0.654	1.579	-0.251						0 0	0.226																										8756				
8766	1 2 3	-0.166	-0.466	0.140						0 0	0.002																										8766				
8891	3 2 1	-0.494	-1.139	-1.270						0 0	-0.334																										8891				
8909	1 3 2																																				8909				
8955	1 3 2	-0.330	-0.305	0.960						0	-0.474																										8955				
9002	1 2 3	0.244	-0.419	0.532	-1.874	0.313				0	-0.278																										9002				
9034	2 3 1	-0.084	-1.348	-1.192	-0.403	-1.049	-0.319			0	0	0.729																									9034				
9051	1 3 2									0		2.465																									9051				
9078	3 2 1	-0.412	-1.279	-0.996						0	-0.670																										9078				
9217	2 1 3	-1.233	-0.721	0.376						0	0.673																										9217				
9408	1 2 3	-2.939	0.046	0.144						0	0.242																										9408				
9429	3 2 1	1.310	-0.303	0.258						0		0.323																									9429				
9436	2 1 3	0.080	-0.140	-0.056	-0.190	-1.058				0	-0.166																										9436				
9441	1 2 3				-1.627	-0.442	-0.200			0		-0.545																									9441				
9453	1 3 2	0.326	-0.489	-1.309						0	-0.446																										9453				
9491	3 2 1									0	-0.726																										9491				
9512	3 2 1									0		0.282																									9512				
9559	3 1 2	0.408	-0.326	-1.975						0	0.282																										9559				
9662	2 3 1	-1.397	-1.162	-1.035						0		2.745																									9662				
9747	1 2 3	1.228	2.833	-1.231						0	0.394																										9747				
9783	2 3 1	1.146	1.555	0.611																																	9783				
9886	1 3 2	-0.248	0.162	0.650	-0.123	0.963	0.569			0	0.058																										9886				
9890	1 3	-0.494	0.162	0.376						0	0.673																										9890				
9903	2 3 1	-0.330	-0.675	-0.643						0	-0.166																										9903				
9950	1 3 2																																				9950				

The results are not evaluated



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

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

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

### **The Swedish 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: <https://www2.slv.se/absint>

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

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

For more information, visit our website: [www.livsmedelsverket.se/en/RM-micro](http://www.livsmedelsverket.se/en/RM-micro)