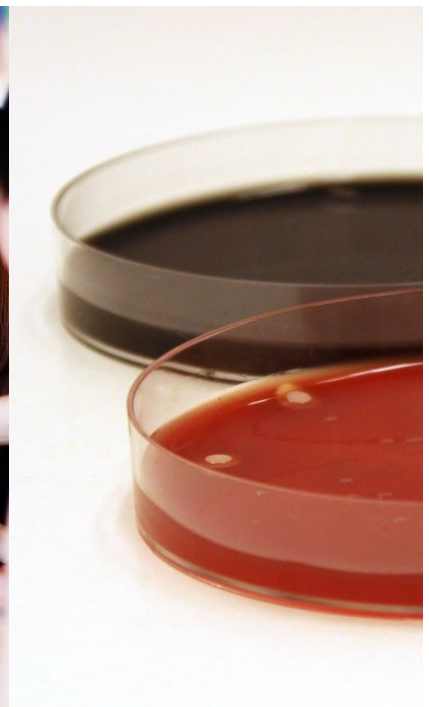


Food Microbiology

April 2021

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Proficiency Testing
Microbiology – Food
April 2021

Quantitative analyses

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

Abbreviations

Media

BA	Blood agar
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
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
MYP	Mannitol egg yolk polymyxin agar
OGYE	Oxytetracyclin glucose yeast extract agar
OPSP	Oleandomycin, Polymixin, Sulphadiazine, Perfringens 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
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
IDF	International Dairy Foundation
ISO	International Organization for Standardization
NMKL	Nordic Committee for Food Analyses
SLV/NFA	Livsmedelsverket/Swedish Food Agency, Sweden

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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 ⁻¹ (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

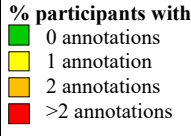
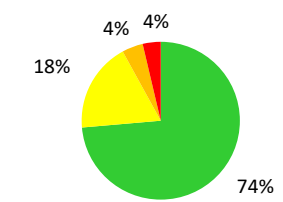
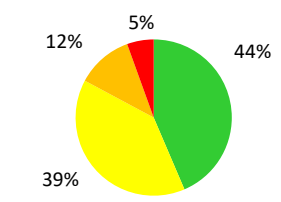
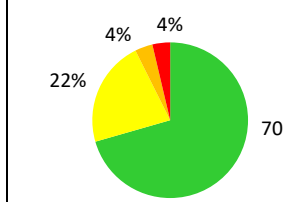
Results of the PT round April 2021

General outcome


Samples were sent to 170 laboratories, 39 in Sweden, 117 in other European countries, and 14 outside of Europe. Of the 163 laboratories that reported results, 112 (69 %) provided at least one result that received an annotation. In the previous round with similar analyses (April 2020) the proportion was 75 %.

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			
% participants with 												
	Microorganisms <i>Bacillus cereus</i> <i>Cladosporium cladosporioides</i> <i>Escherichia coli</i> <i>Kluyveromyces marxianus</i> <i>Lactobacillus plantarum</i> <i>Staphylococcus xylosum</i>				<i>Aeromonas caviae</i> <i>Candida glabrata</i> <i>Clostridium perfringens</i> <i>Penicillium verrucosum</i> <i>Staphylococcus aureus</i>				<i>Aeromonas hydrophila</i> <i>Clostridium bifermentans</i> <i>Escherichia coli</i> <i>Hafnia alvei</i> <i>Lactobacillus plantarum</i> <i>Staphylococcus aureus</i>			
Analysis	Target	N	F	X	Target	N	F	X	Target	N	F	X
Aerobic micro-organisms, 30 °C	All	147	0 %	2 %	All	149	0 %	4 %	All	148	0 %	3 %
Psychrotrophic microorganisms	All	20	0 %	0 %	All	21	0 %	0 %	All	21	0 %	5 %
Enterobacteriaceae	<i>E. coli</i>	128	2 %	3 %	(<i>A. caviae</i>)	128	32 %	0 %	<i>E. coli</i> <i>H. alvei</i>	130	2 %	2 %
<i>Escherichia coli</i>	<i>E. coli</i>	113	6 %	3 %	-	116	1 %	0 %	<i>E. coli</i>	114	2 %	3 %
Presumptive <i>B. cereus</i>	<i>B. cereus</i>	113	6 %	6 %	(<i>A. caviae</i>) (<i>S. aureus</i>)	115	2 %	0 %	(<i>A. hydrophila</i>) (<i>S. aureus</i>)	115	6 %	0 %
Coagulase-positive staphylococci	(<i>S. xylosum</i>)	97	7 %	0 %	<i>S. aureus</i>	99	4 %	4 %	<i>S. aureus</i>	99	5 %	7 %
Lactic acid bacteria	<i>L. plantarum</i>	56	0 %	5 %	(<i>C. glabrata</i>) (<i>S. aureus</i>)	55	42 %	0 %	<i>L. plantarum</i>	56	0 %	9 %
<i>C. perfringens</i>	-	58	0 %	0 %	<i>C. perfringens</i>	59	5 %	3 %	(<i>C. bifermentans</i>)	59	19 %	0 %
Anaerobic sulphite-reducing bacteria	-	61	2 %	0 %	<i>C. perfringens</i>	59	2 %	2 %	<i>C. bifermentans</i>	59	5 %	5 %
Aerobic microorg. in fish products	All	27	0 %	0 %	All	28	0 %	4 %	All	28	0 %	4 %
H ₂ S-prod. bacteria in fish products	-	24	4 %	0 %	-	25	8 %	0 %	<i>H. alvei</i>	25	8 %	4 %
Yeasts	<i>K. marxianus</i>	132	2 %	5 %	<i>C. glabrata</i>	132	2 %	6 %	-	133	5 %	0 %
Moulds	<i>C. cladosporioides</i>	129	8 %	3 %	<i>P. verrucosum</i>	129	24 %	3 %	-	130	2 %	0 %

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

 Not evaluated

Aerobic microorganisms 30 °C

Sample A

The strains of *B. cereus*, *L. plantarum* and *E. coli* were present in the highest concentrations and were thus the main target organisms.

Three low outliers were reported.

Sample B

The strains of *S. aureus* and *A. caviae* were present in the highest concentrations and were thus the main target organisms.

Four low and two high outliers were reported.

Sample C

The strains of *E. coli*, *L. plantarum* and *A. hydrophila* were present in the highest concentrations and were thus the main target organisms.

Three low and two high outliers were reported.

General remarks

As in previous proficiency testing rounds most laboratories used NMKL 86:2013 (30 %), ISO 4833-1:2013 (22 %) or Petrifilm AC (17 %). However the older NMKL 86:2006 (7 %) and ISO 4833:2003 (4 %) were also used. Both NMKL 86 and ISO 4833 are based on incubation on PCA or MPCA at 30 °C for 72 h. Users of Petrifilm AC can use different times/temperatures, 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. ISO 4833-1:2013 was last reviewed by ISO in 2019 and remains current.

The majority of the laboratories incubated on 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.

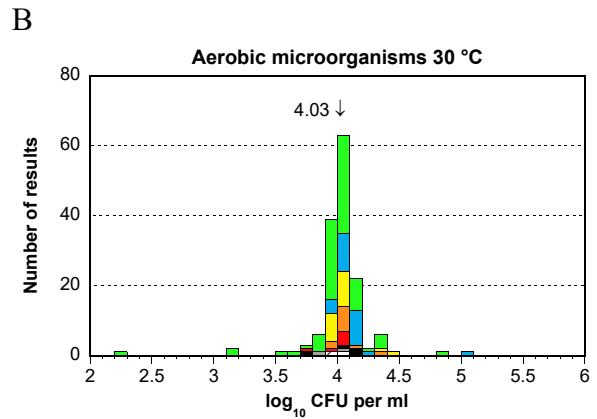
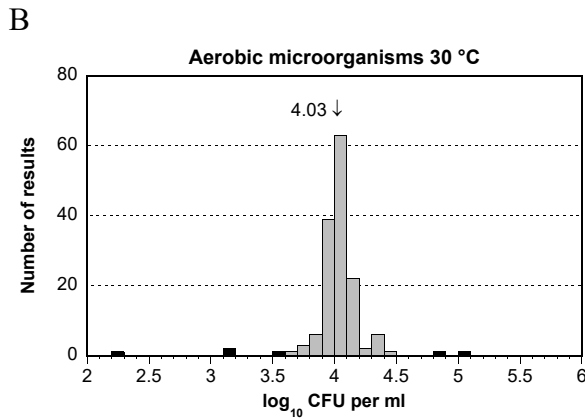
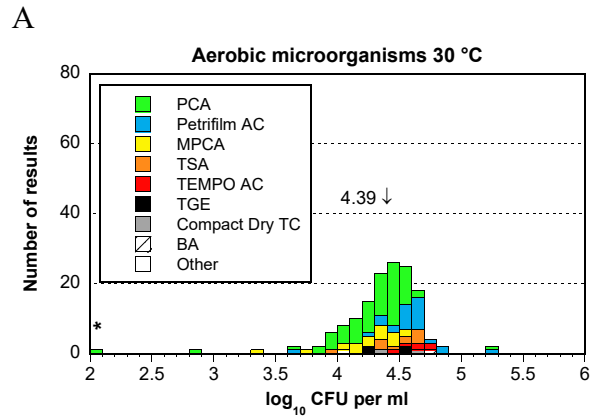
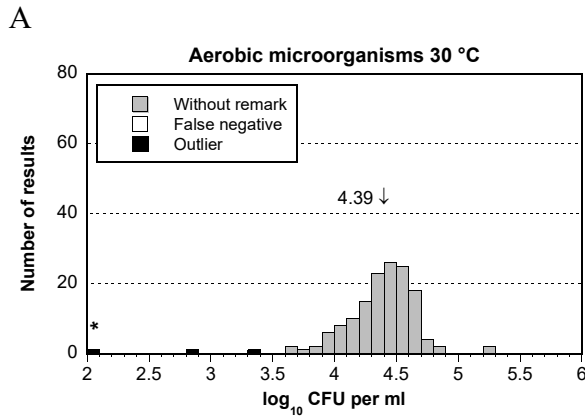
Five laboratories used TEMPO AC, which is based on MPN (Most Probable Number). With this method, the sample is incubated in a card that contains different-sized wells. A substrate in the medium emits fluorescence when hydrolysed by the microorganisms. The number of microorganisms is determined statistically by the number and size of the fluorescing wells.

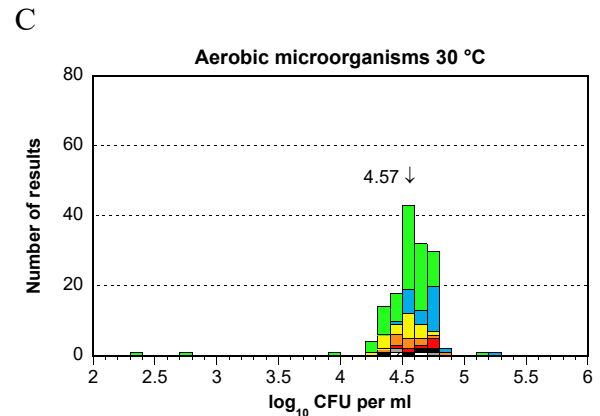
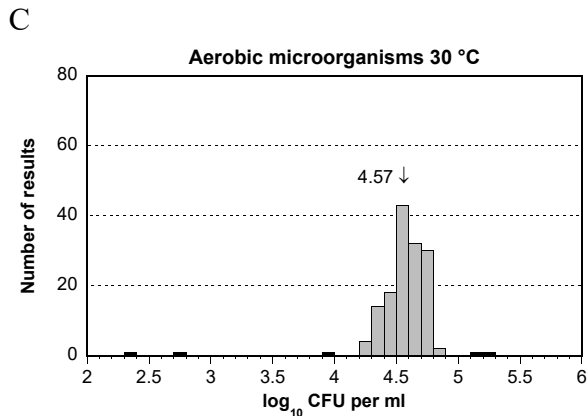
The results for the different method and media were very similar and no significant differences could be identified.

Comment: One laboratory stated following ISO 4832:2006 (coliform bacteria), and another stated ISO 13559/IDF 153 (contaminating microorganisms). However both of these laboratories used media suited for the analysis of aerobic microorganisms.

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	147	144	4.39	0.26	0 3 0	149	143	4.03	0.12	0 4 2	148	143	4.57	0.13	0 3 2
PCA	75	73	4.31	0.24	0 2 0	77	72	4.02	0.13	0 4 1	76	72	4.55	0.12	0 3 1
Petrifilm AC	27	27	4.57	0.27	0 0 0	27	26	4.09	0.07	0 0 1	27	26	4.67	0.10	0 0 1
MPCA	20	19	4.27	0.19	0 1 0	20	20	4.04	0.13	0 0 0	20	20	4.51	0.14	0 0 0
TSA	11	11	4.46	0.22	0 0 0	11	11	4.05	0.10	0 0 0	11	11	4.55	0.15	0 0 0
TEMPO AC	6	6	4.61	0.10	0 0 0	6	6	4.00	0.12	0 0 0	6	6	4.65	0.13	0 0 0
TGE	4	4	-	-	0 0 0	4	4	-	-	0 0 0	4	4	-	-	0 0 0
Compact Dry TC	2	2	-	-	0 0 0	2	2	-	-	0 0 0	2	2	-	-	0 0 0
BA	1	1	-	-	0 0 0	1	1	-	-	0 0 0	1	1	-	-	0 0 0
Other	1	1	-	-	0 0 0	1	1	-	-	0 0 0	1	1	-	-	0 0 0





Psychrotrophic microorganisms

Sample A

The strains of *B. cereus*, *L. plantarum* and *E. coli* were present in the highest concentrations and were thus the main target organisms. In the quality control at the Swedish Food Agency (ten days incubation on PCA at 6.5 °C), a larger than expected variation was found among the results, which meant that the requirements for homogeneity were not met. Therefore, no results are considered as outliers. Laboratories that reported results lower than 3.0 log₁₀ cfu ml⁻¹ are however encouraged to repeat the analysis.

In total, 18 positive results and two zero results were reported. Laboratories that incubated at 6.5 °C reported lower results than those that incubated at higher temperatures.

Due to the difficulties, the results for sample A are not evaluated further, and no z-scores are calculated for the analysis.

Sample B

The strains of *S. aureus* and *A. caviae* were present in the highest concentrations and were thus the main target organisms. In the quality control at the Swedish Food Agency, the requirements for homogeneity were not met.

In total, 18 positive results and three zero results were reported. The results had a wide distribution, and it cannot be ruled out that zero results were obtained purely by chance.

Due to the difficulties, the results for sample B are not evaluated further, and no z-scores are calculated for the analysis.

Sample C

The strains of *E. coli*, *L. plantarum* and *A. hydrophila* were present in the highest concentrations and were thus the main target organisms.

One low outlier was reported. Laboratories that incubated at 21 °C reported higher results than those that incubated at lower temperatures.

General remarks

In total, 21 laboratories performed the analysis. The majority of these (71 %) incubated on PCA, but MPCA (14 %) and Petrifilm AC (10 %) were also used. One laboratory incubated on Long & Hammer agar.

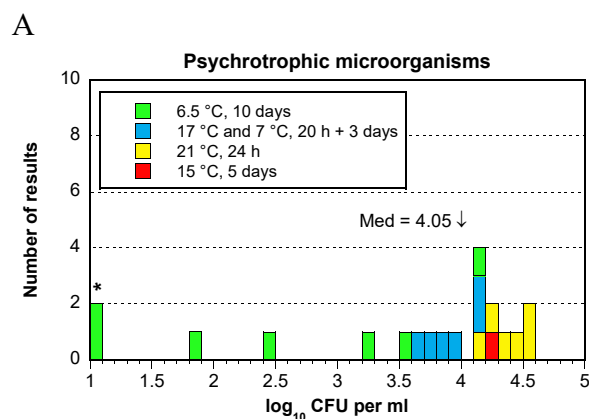
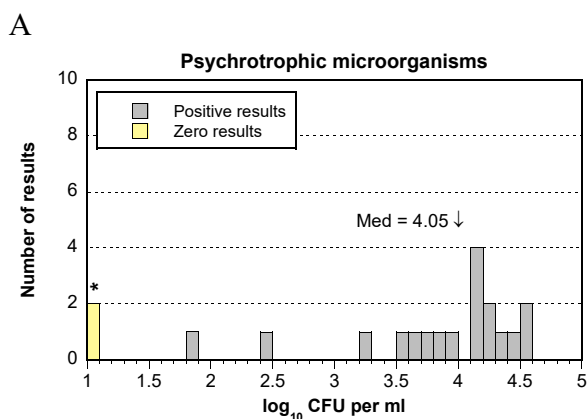
As before, there were considerable variations in the conditions for incubation, due to differences in the methods used by the laboratories. NMKL 86:2013 prescribes 10 days at 6.5 °C, but 20 h at 17 °C followed by 3 days at 7 °C can also be used. For psychrotrophic microorganisms in milk, ISO 6730:2005/IDF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

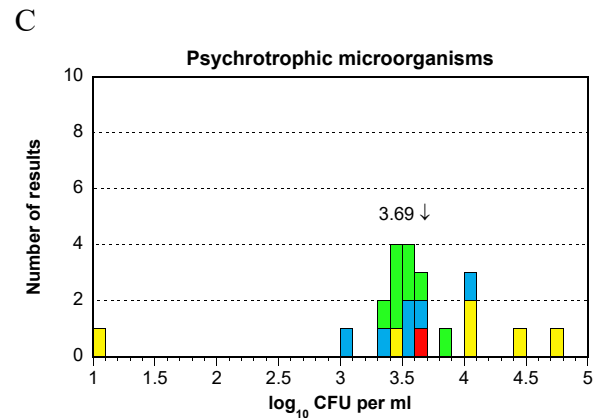
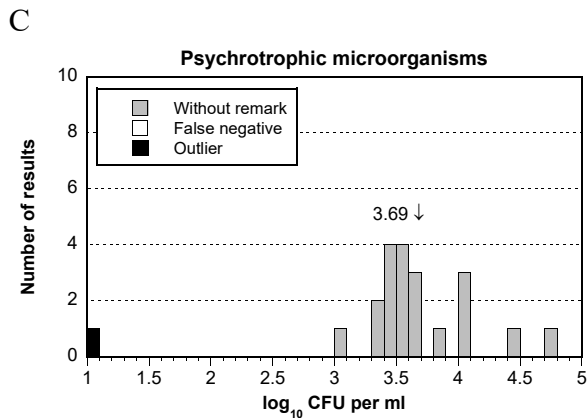
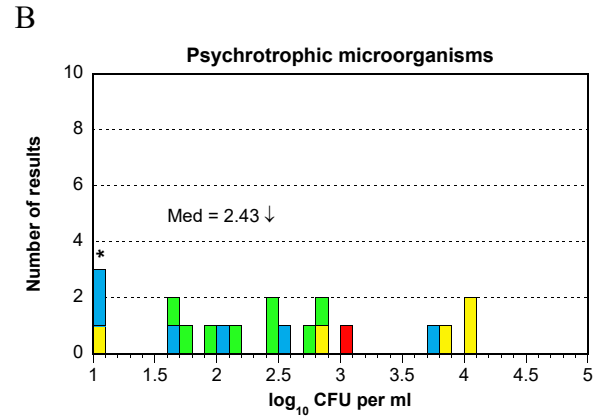
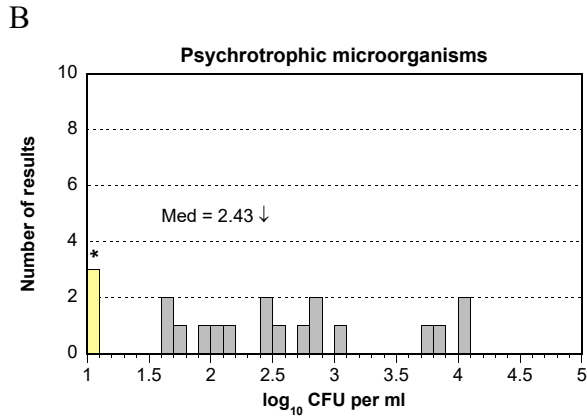
The low number of participants makes it difficult to see if the false negative results are due to using a specific method or medium. The results are therefore difficult to evaluate, especially since a few laboratories also stated temperatures, incubation times or media that did not match the method they used. The majority of the methods could however be divided into three groups. In general, 21 °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	Med*	s	F	<	>	N	n	m	s	F	<	>
All results	20	20	4.05	1.36	0	0	0	21	21	2.43	1.30	0	0	0	21	20	3.69	0.41	0	1	0
6,5 °C, 10 days	7	7	2.48	1.67	0	0	0	8	8	2.29	0.46	0	0	0	8	8	3.52	0.15	0	0	0
17 and 7 °C, 20 h + 3 days	6	6	3.92	0.18	0	0	0	6	6	1.80	1.48	0	0	0	6	6	3.53	0.33	0	0	0
21 °C, 24 h	6	6	4.39	0.15	0	0	0	6	6	3.37	1.93	0	0	0	6	5	4.15	0.49	0	1	0
15 °C, 5 days	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

* Med = median





Enterobacteriaceae

Sample A

The strain of *E. coli* was target organism. In the Swedish Food Agency's quality control on VRBG, it formed typical red/purple colonies surrounded by a bile salt precipitation zone.

Three low and one high outliers were reported, as well as two false negative results.

Sample B

No target organism was present in the sample, but the strain of *A. caviae* may form red colonies on VRBG. It can however be distinguished from Enterobacteriaceae since it is oxidase-positive. The strain of *A. caviae* was present in approximately 3.5 log₁₀ cfu ml⁻¹ in the sample.

A total of 41 false positive results were reported. These were distributed relatively evenly between 0.3 and 3.4 log₁₀ cfu ml⁻¹, with a possible small peak at 2.0 log₁₀ cfu ml⁻¹. The false positive results were distributed relatively evenly among the different methods and media that were used, though a slight over-representation was found for laboratories that used TEMPO EB or Petrifilm EB.

Sample C

The strains of *E. coli* and *H. alvei* were target organisms. On VRBG, they form typical pink/red colonies with a bile salt precipitation zone.

In the Swedish Food Agency's quality control on VRBG, small colonies without a precipitation zone were also observed. Upon confirmation, these were oxidase-positive, and they were therefore not considered as Enterobacteriaceae. They are instead assumed to be *A. hydrophila*, which is also present in the sample.

Two low outliers were reported, as well as two false negative results.

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 salt 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 (25 %), while the ISO methods (various versions) were used by 19 %. ISO 21528-2:2017 is based on colony-count, while ISO 21528-1:2017 is based on MPN. The latter method is recommended when the expected level of Enterobacteriaceae is lower than 100 cfu g⁻¹.

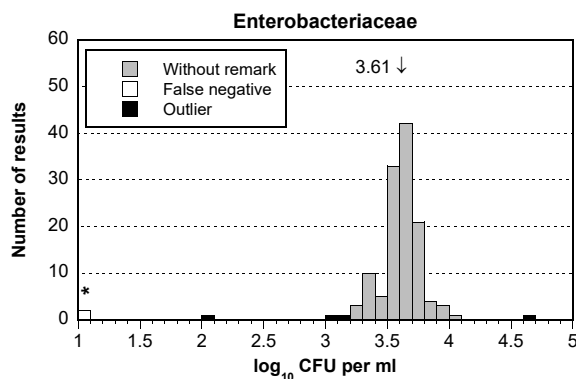
The number of users of ISO 21528-2:2017 was higher compared to ISO 21528-2:2004 (15 and 5 laboratories, respectively). In comparison, four laboratories stated the older ISO 21528-1:2004, while only one stated the new ISO 21528-1:2017.

NMKL 144:2005 stipulates confirmation of presumptive colonies 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. Here, the majority of the laboratories that performed a confirmation test, specified that this consisted of an oxidase test.

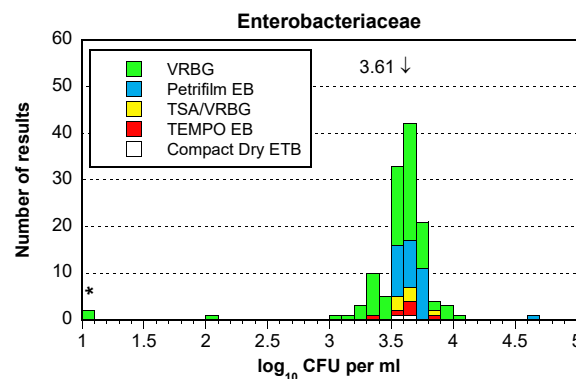
Results from analysis of Enterobacteriaceae

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	128	122	3.61	0.14	2	3	1	128	87	-	-	41	-	-	130	126	4.09	0.17	2	2	0
VRBG	80	75	3.59	0.16	2	3	0	80	59	-	-	21	-	-	81	78	4.06	0.17	1	2	0
Petrifilm EB	33	32	3.64	0.08	0	0	1	32	19	-	-	13	-	-	33	32	4.15	0.16	1	0	0
TSA/VRBG	7	7	3.63	0.12	0	0	0	8	6	-	-	2	-	-	8	8	4.14	0.17	0	0	0
TEMPO EB	6	6	3.62	0.16	0	0	0	6	2	-	-	4	-	-	6	6	4.08	0.12	0	0	0
Compact Dry ETB	2	2	-	-	0	0	0	2	1	-	-	1	-	-	2	2	-	-	0	0	0

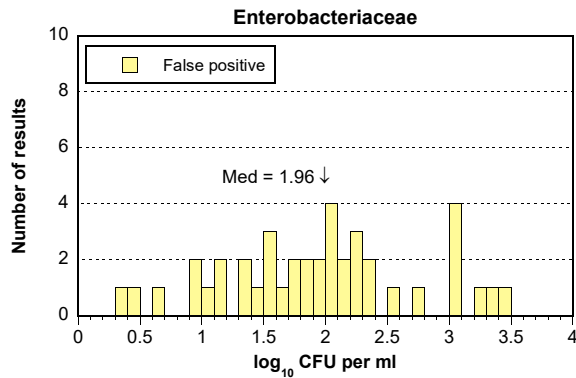
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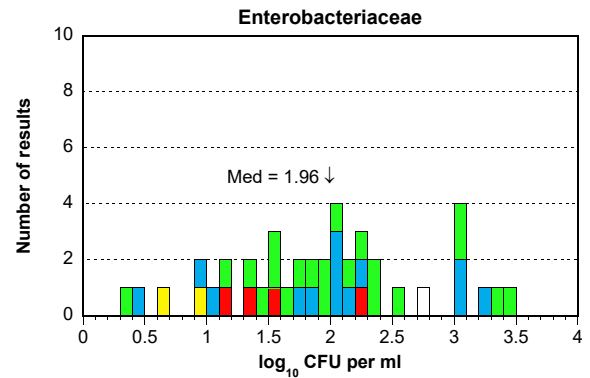
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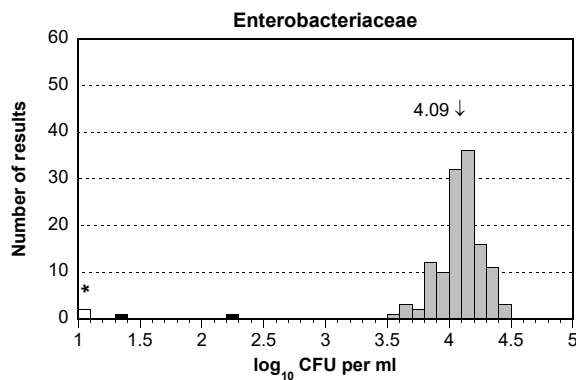
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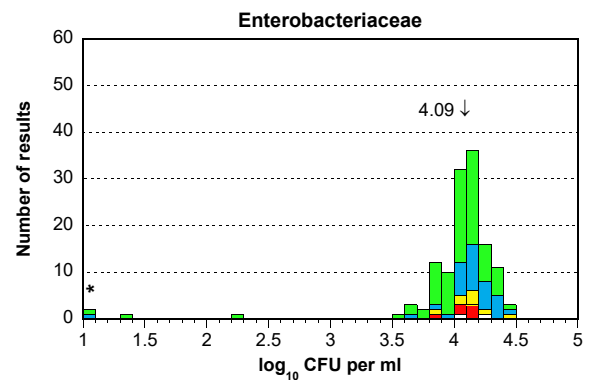
B



C



C



Escherichia coli

Sample A

The strain of *E. coli* was target organism. On TSA/VRB, it 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/VRG. Upon confirmation, the strain of *E. coli* produces both gas and indole in LTL SB. It is also positive for β -glucuronidase.

Two low and one high outliers were reported, as well as seven false negative results. The false negative results could not be attributed to the use of a specific method or medium.

Sample B

No target organism was present in the sample.

One false positive result was reported.

Sample C

The strain of *E. coli* (not identical to that in sample A) was target organism for the analysis. On TSA/VRB, it forms typical dark red colonies surrounded by a red precipitation zone. The strain is positive for indole production and β -glucuronidase activity, and it produces gas in LTL SB.

Two low and one high outliers were reported, as well as two false negative results.

General remarks

In total, 34 % of the laboratories used a method based on 3M™ Petrifilm™. NMKL 125:2005 and ISO 16649-2:2001 were in comparison used by 28 % and 15 % 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 incubated on Petrifilm EC/CC or Petrifilm SEC. It can also be mentioned that NMKL 125 is currently undergoing revision, and the new version will likely be more similar to ISO 16649-2.

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.

The definition of *E. coli* differs between the methods. ISO 16649-2:2001 defines *E. coli* as bacteria that form typical blue (i.e. β -glucuronidase positive) colonies on TBX, with no additional confirmation. Petrifilm EC/CC and Petrifilm SEC are also based on media that detect *E. coli* β -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, and that also produce gas as a result of lactose fermentation. Thermotolerant coliform bacteria that also produce indole are considered to be *E. coli*.

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. Overall, relatively more false results were reported by laboratories that did not perform a confirmation. 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 in sample C, compared to other media. Similar differences have been observed in several previous proficiency testing rounds, and can therefore be considered normal.

Due to method differences, incubation was either at 41.5–44 °C (57 %) or at 35–37 °C (43 %). The mean values, and the number of false results and outliers did not differ notably for the two temperature groups, neither for samples A nor for sample B. However for sample C, all false results and outliers were reported by laboratories that incubated at 41.5–44 °C. Still, the mean values for the two temperature groups did not differ.

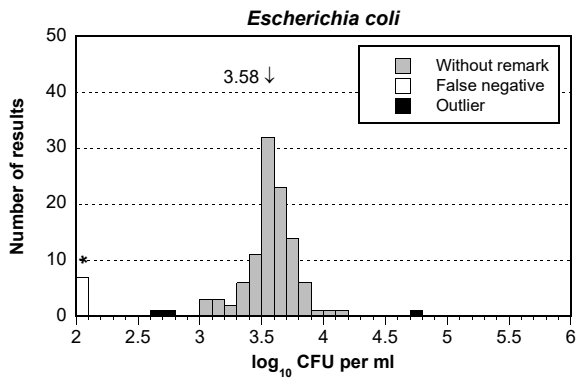
Results from analysis of *Escherichia coli*

Medium	Sample A						Sample B						Sample C					
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >
All results	113	103	3.58	0.19	7	2 1	116	115	-	-	1	- -	114	109	3.93	0.24	2	2 1
Petrifilm EC/CC	22	22	3.57	0.16	0	0 0	23	23	-	-	0	- -	23	23	4.00	0.15	0	0 0
TSA/VRB*	21	20	3.61	0.09	1	0 0	22	22	-	-	0	- -	22	22	4.07	0.20	0	0 0
TBX	21	19	3.45	0.17	1	1 0	21	21	-	-	0	- -	21	21	3.66	0.19	0	0 0
Petrifilm SEC	18	15	3.63	0.10	2	0 1	18	18	-	-	0	- -	18	15	3.99	0.09	2	0 1
VRB	7	6	3.65	0.15	1	0 0	7	7	-	-	0	- -	7	6	4.05	0.09	0	1 0
EC	4	4	-	-	0	0 0	4	4	-	-	0	- -	3	2	-	-	0	1 0
Rapid'E.coli 2	4	4	-	-	0	0 0	4	3	-	-	1	- -	4	4	-	-	0	0 0
TEMPO EC	4	4	-	-	0	0 0	4	4	-	-	0	- -	4	4	-	-	0	0 0
Other**	12	9	-	-	2	1 0	13	13	-	-	0	- -	12	12	-	-	0	0 0

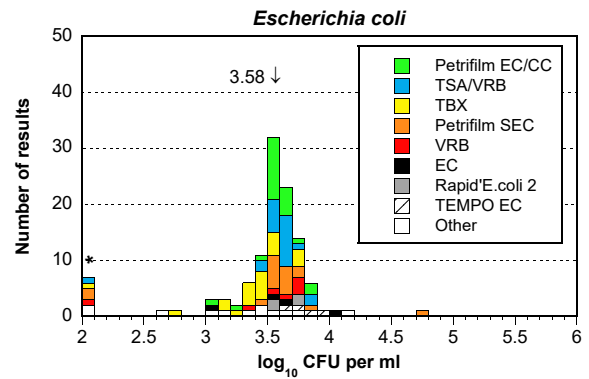
** Includes three laboratories that used TSA/VRBG.

* Includes Brilliance EC/CC, Compact Dry EC/CC, CHROMID®, ECC ChromoSelect Selective Agar and Rebecka agar.

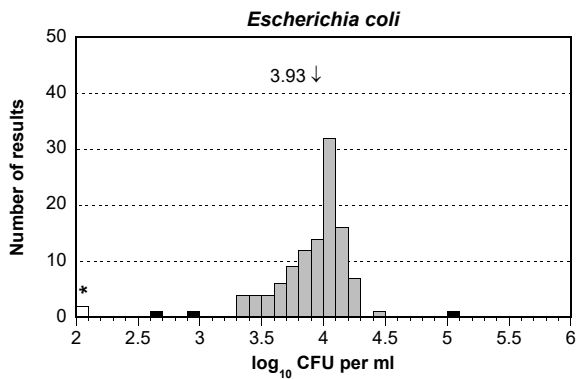
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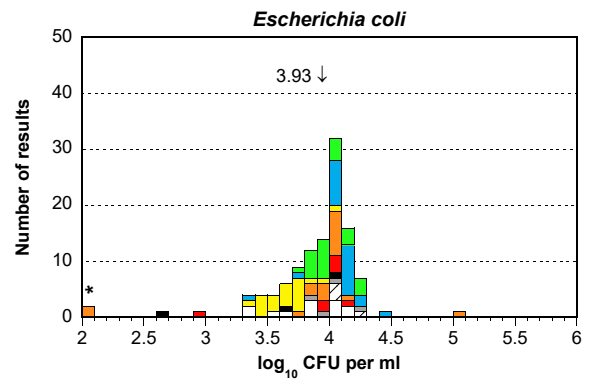
A



C



C



Presumptive *Bacillus cereus*

Sample A

The strain of *B. cereus* was target organism, but the strains of *E. coli*, *L. plantarum* and *S. xyloso* that are present in the sample may also form colonies on BA. In the Swedish Food Agency's quality control on BA, the strain of *B. cereus* formed typical colonies surrounded by a zone of haemolysis. On BcsA, it formed typical blue colonies surrounded by a precipitation zone.

Six low and one high outliers were reported, as well as seven false negative results. These were relatively evenly distributed among the different methods and media.

Sample B

No target organism was present in the sample. The strains of *S. aureus* and *A. caviae* may however form atypical colonies on BA. In the Swedish Food Agency's quality control, two types of atypical white colonies without a zone of haemolysis were also observed on BA. Upon confirmation on BcsA, neither displayed any blue colour.

Two false positive results were reported.

Sample C

No target organism was present in the sample. Several strains in the sample may however form atypical colonies on BA. *A. hydrophila* and *S. aureus* may form atypical colonies also on BcsA, which could explain the presence of false positive results.

Seven false positive results were reported. All were reported by laboratories that followed NMKL 67:2010. However, four of the seven laboratories stated that they only incubated on BA.

General remarks

Most laboratories followed either NMKL 67:2010 (51 %) or ISO 7932:2004 (26 %), 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. In comparison, ISO 7932:2004 prescribes incubation on MYP, which is followed by confirmation of haemolysis on BA. An amendment was recently published for the ISO method (ISO 7932:2004/Amd 1:2020). It contains optional tests, including for PCR detection of *cytK* genes. A new version of NMKL 67 will be published in 2021; it will include fundamental changes in the choice of media and incubation.

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 MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity.

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

As in previous proficiency testing rounds, the reporting of method data for *B. cereus* was in several cases ambiguous, or difficult to interpret. For example, several laboratories reported combinations of method and media that are incompatible. Despite

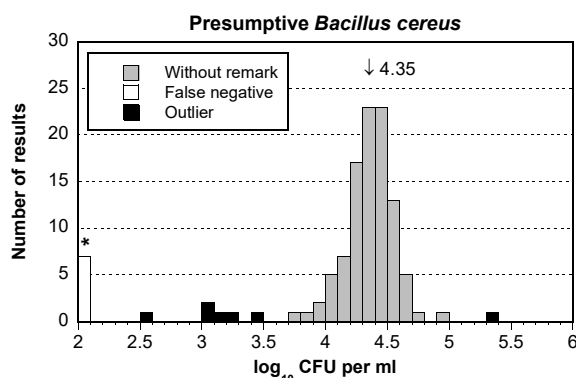
these uncertainties, the results and mean values for the different methods and media were very similar.

Results from analysis of presumptive *Bacillus cereus*

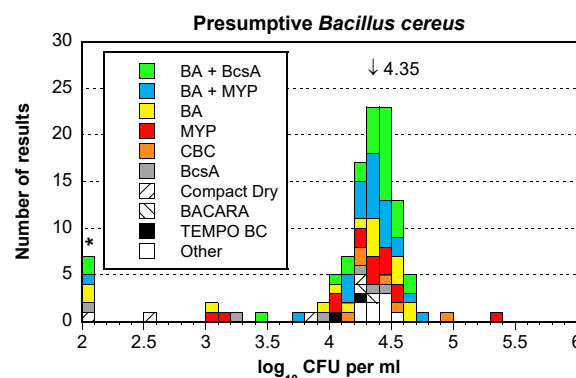
Medium	Sample A					Sample B					Sample C				
	N	n	m	s	F < >	N	n	m	s	F < >	N	n	m	s	F < >
All results	113	99	4.35	0.19	7 6 1	115	113	-	-	2 - -	115	108	-	-	7 - -
BA + BcsA	29	26	4.40	0.15	2 1 0	30	29	-	-	1 - -	30	28	-	-	2 - -
BA + MYP	25	24	4.35	0.21	1 0 0	26	26	-	-	0 - -	26	25	-	-	1 - -
BA	16	13	4.35	0.22	2 1 0	16	16	-	-	0 - -	16	12	-	-	4 - -
MYP	15	12	4.33	0.16	0 2 1	15	15	-	-	0 - -	15	15	-	-	0 - -
CBC	6	6	4.42	0.28	0 0 0	6	6	-	-	0 - -	6	6	-	-	0 - -
BcsA	6	4	-	-	1 1 0	6	6	-	-	0 - -	6	6	-	-	0 - -
Compact Dry X-BC	4	2	-	-	1 1 0	4	3	-	-	1 - -	4	4	-	-	0 - -
BACARA™	2	2	-	-	0 0 0	2	2	-	-	0 - -	2	2	-	-	0 - -
TEMPO BC	2	2	-	-	0 0 0	2	2	-	-	0 - -	2	2	-	-	0 - -
Other*	8	8	-	-	0 0 0	8	8	-	-	0 - -	8	8	-	-	0 - -

* Includes COMPASS® *Bacillus cereus* agar, Brilliance™ *Bacillus cereus* and PEMBA.

A



A



Coagulase-positive staphylococci

Sample A

No target organism was present in the sample. The coagulase-negative strain of *S. xylosus* was however present as a false-positive organism for the analysis. In the Swedish Food Agency's quality control on RPFA, it formed atypical colonies without a precipitation zone.

Seven false positive results were reported. The concentrations of the reported results suggest that the participants have counted colonies of *S. xylosus*.

Sample B

The strain of *S. aureus* was target organism. On RPFA, it forms typical colonies surrounded by a precipitation zone.

Two low and two high outliers were reported, as well as four false negative results.

Sample C

The strain of *S. aureus* (not identical to that in sample B) was target organism. On RPFAs, it forms typical colonies surrounded by a precipitation zone.

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

General remarks

Most laboratories (44 %) followed NMKL 66:2009. Other major methods were 3M™ Petrifilm™ (13 %), ISO 6888-1:1999 (13 %) and ISO 6888-2:1999 (7 %). Both ISO 6888-1:1999 and ISO 6888-2:1999 were last reviewed by ISO in 2015 and remain current. An alternative confirmation by stab-culture in RPFAs was added in 2018 for ISO 6888-1 (ISO 6888-1:1999/Amd 2:2018). One laboratory followed the MPN-based ISO 6888-3:2003, which is adapted for use when low numbers of stressed coagulase-positive staphylococci are expected.

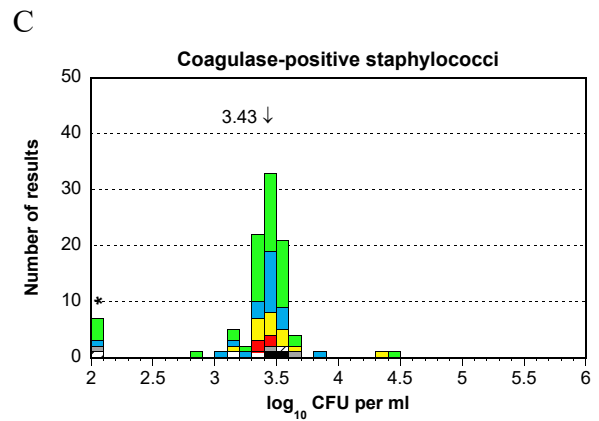
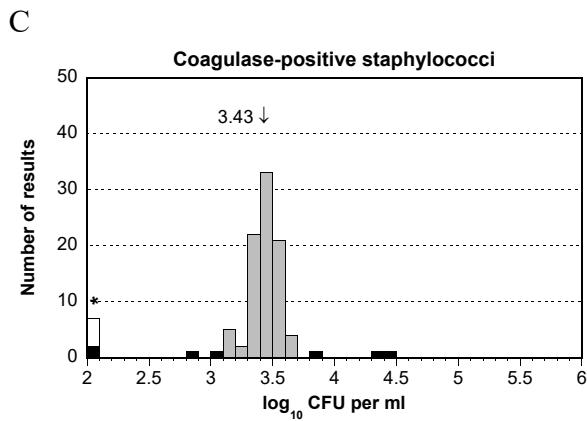
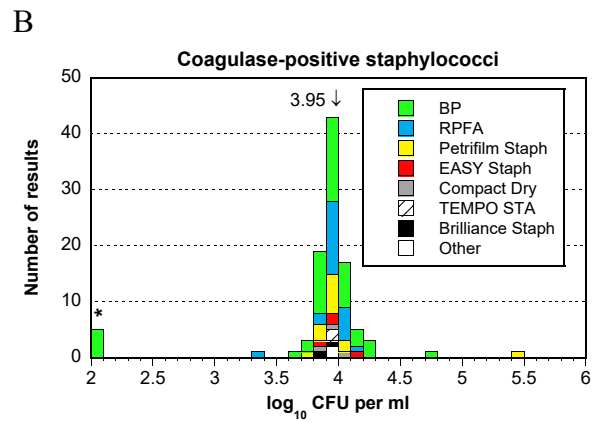
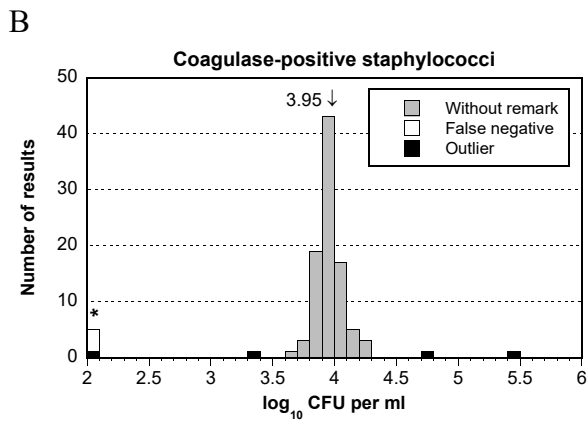
With NMKL 66:2009 incubation is done on BP and/or RPFAs. In comparison, ISO 6888-1:1999 stipulates surface spreading on BP, whereas 6888-2:1999 stipulates the use of RPFAs. On BP, *S. aureus* form characteristic convex, shiny colonies that have a grey/black colour due to reduction of tellurite in the 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. With BP, colonies are usually confirmed by a positive result in a coagulase test. With RPFAs, the coagulase activity is instead tested directly in the medium. 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, RPFAs and Petrifilm Staph, in all three samples. Outliers and false results could not be attributed to the use of a specific method, medium or confirmation test. Somewhat lower mean values have in previous proficiency testing rounds sometimes been seen for Petrifilm Staph, but this was not evident this time. The media EASY Staph®, Compact Dry™ X-SA, TEMPO STA and Brilliance™ Staph 24 were only used by a small number of laboratories, which makes them difficult to evaluate.

In total, 73 % of the laboratories stated that they performed some kind of confirmation. When using BP, this usually consisted of a tube coagulase test, while users of Petrifilm Staph mainly used Petrifilm Disk for confirmation. 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	97	90	-	-	7	- -	99	91	3.95	0.10	4	2 2	99	87	3.43	0.11	5	4 3
BP	48	44	-	-	4	- -	49	43	3.95	0.12	4	1 1	49	43	3.43	0.11	3	2 1
RPFA	22	21	-	-	1	- -	23	22	3.96	0.06	0	1 0	23	20	3.44	0.10	1	1 1
Petrifilm Staph	14	12	-	-	2	- -	14	13	3.92	0.07	0	0 1	14	13	3.43	0.13	0	0 1
EASY Staph	4	4	-	-	0	- -	4	4	-	-	0	0 0	4	4	-	-	0	0 0
Compact Dry X-SA	3	3	-	-	0	- -	3	3	-	-	0	0 0	3	2	-	-	1	0 0
TEMPO STA	2	2	-	-	0	- -	2	2	-	-	0	0 0	2	1	-	-	0	1 0
Brilliance Staph 24	2	2	-	-	0	- -	2	2	-	-	0	0 0	2	2	-	-	0	0 0
Other	2	2	-	-	0	- -	2	2	-	-	0	0 0	2	2	-	-	0	0 0



Lactic acid bacteria

Sample A

The strain of *L. plantarum* was target organism. In the Swedish Food Agency's quality control on MRS-aB, it formed typical shiny round white colonies. The strain is Gram-positive and catalase-negative. The strains of *B. cereus* and *S. xylosus* may form small colonies on MRS-aB. They can however be distinguished with a catalase test.

One low and two high outliers were reported.

Sample B

No target organism was present in the sample, but the strains of *C. glabrata* and *S. aureus* may sometimes form colonies on the media most commonly used for this analysis. In the Swedish Food Agency's quality control, no colonies were however observed on MRS-aB.

In total, 23 false positive results were reported, to which there is no single obvious explanation. The false positive results were relatively evenly distributed between 1.3 and 4.0 log₁₀ cfu ml⁻¹, with a median of 3.0 log₁₀ cfu ml⁻¹. This suggests that it is possibly *C. glabrata* that has been detected. However, four of the six laboratories that stated they used confirmation with microscopy still reported a false positive result. There was also no obvious difference in the results between laboratories that used Gram staining and/or a catalase test for confirmation, and those that did not. Users of NMKL 140:2007 reported relatively fewer false positive results, while users of the older NMKL 140:1991 and users of 3M Petrifilm (LAB) reported relatively more. The differences are however small.

Sample C

The strain of *L. plantarum* (not identical to that in sample A) was target organism. In the Swedish Food Agency's quality control on MRS-aB, it formed typical round white colonies. The strain is catalase-negative.

Four low and one high outliers were reported. Laboratories that incubated on Rogosa agar (adapted for *Lactobacillus*) on average reported higher results compared to laboratories that used other media.

General remarks

Most of the laboratories followed NMKL 140, either NMKL 140:2007 (36 %), or the older NMKL 140:1991 (9 %). 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. ISO 15214:1998 was reviewed by ISO in 2021, and remains current. The number of users of Petrifilm LAB has increased the last few proficiency testing rounds, and the method was here used by 15 % of the laboratories. Two laboratories stated ISO 7889 / IDF 117, which is a method for characteristic microorganisms in yoghurt at 37 °C.

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.

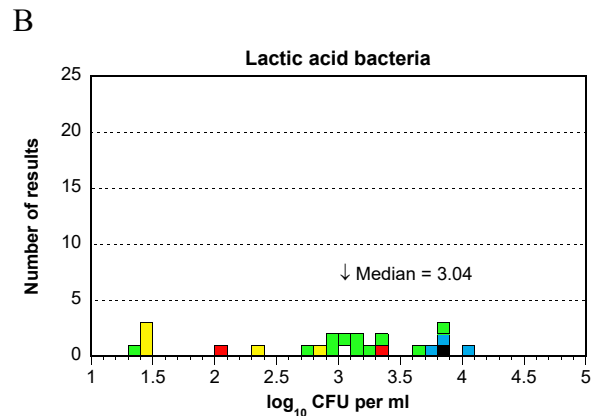
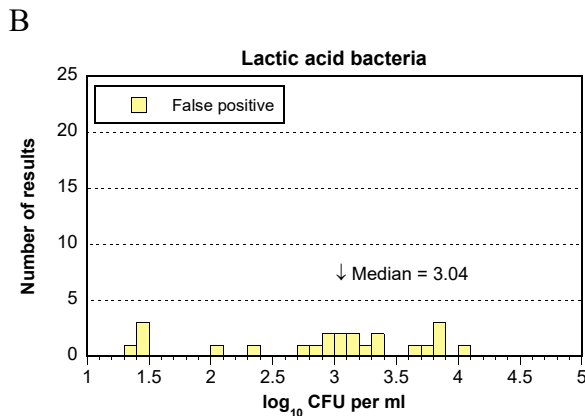
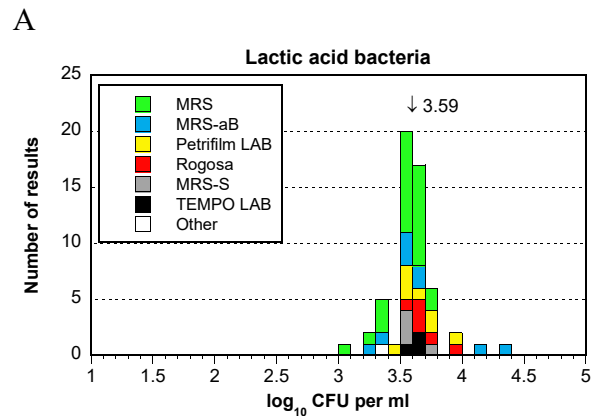
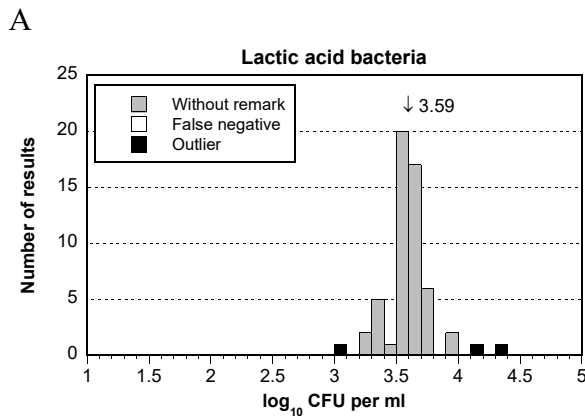
Lactic acid bacteria constitute a heterogeneous group of microorganisms, and therefore have different optimal medium, pH and incubation conditions. For example, MRS-aB

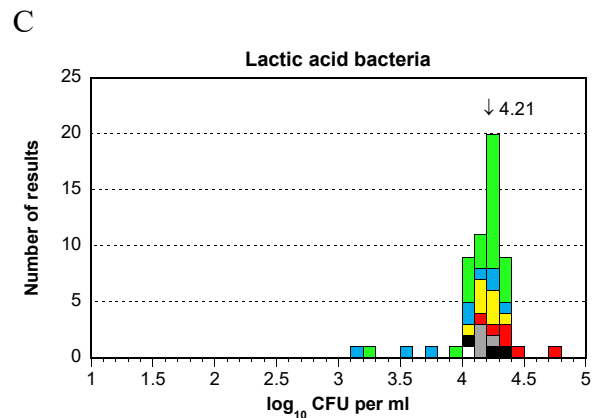
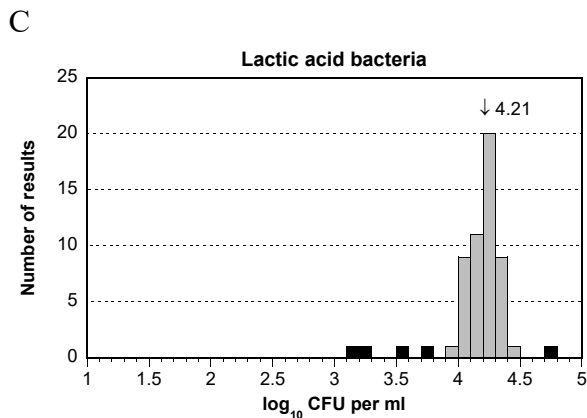
(pH 6.2) is a less selective medium that allows the growth of a more wide range of lactic acid bacteria. This may however also result in the appearance of more false positive colonies compared to the more acid media MRS and MRS-S (pH 5.7). Such differences between media and incubation conditions underline the importance of performing a confirmation test in uncertain cases, especially when using a less selective medium.

Both the ISO and the NMKL methods recommend confirmation of uncertain colonies by Gram staining and/or with a catalase test. Lactic acid bacteria are Gram positive and normally catalase-negative. Confirmation was in this proficiency testing performed by roughly half (53 %) of the laboratories. Usually, it consisted of a catalase test. As a whole, the use of a confirmation test does not appear to have had an impact on the result.

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	56	53	3.59	0.14	0 1 2	55	32	-	-	23 - -	56	51	4.21	0.10	0 4 1
MRS	25	24	3.57	0.13	0 1 0	24	13	-	-	11 - -	25	24	4.21	0.10	0 1 0
MRS-aB	9	7	3.50	0.17	0 0 2	9	6	-	-	3 - -	9	6	4.19	0.10	0 3 0
Petrifilm LAB	8	8	3.65	0.16	0 0 0	8	3	-	-	5 - -	8	8	4.20	0.08	0 0 0
Rogosa	6	6	3.68	0.13	0 0 0	6	4	-	-	2 - -	6	5	4.32	0.12	0 0 1
MRS-S	4	4	-	-	0 0 0	4	4	-	-	0 - -	4	4	-	-	0 0 0
TEMPO LAB	3	3	-	-	0 0 0	3	2	-	-	1 - -	3	3	-	-	0 0 0
Other	1	1	-	-	0 0 0	1	0	-	-	1 - -	1	1	-	-	0 0 0





Clostridium perfringens

Sample A

No target organism was present in the sample.

No false positive results were reported.

Sample B

The strain of *C. perfringens* was target organism. On TSC, it forms typical black colonies. The strain is non-motile, and ferments lactose. It does not form colonies on BA that is incubated aerobically.

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

Sample C

No target organism was present in the sample. It did however contain a strain of *C. bifermentans*, which is false positive for the analysis. It can be distinguished from *C. perfringens* after confirmation, for example since *C. bifermentans* is motile.

Eleven false positive results were reported. The concentrations of the reported results suggest that the participants have counted *C. bifermentans*, despite the fact that six of the eleven laboratories performed a confirmation with a motility test.

General remarks

The majority of the laboratories (69 %) followed NMKL 95:2009. One laboratory followed the older NMKL 95:1997. ISO 7937:2004 was followed by 22 % of the laboratories. Two laboratories 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 (86 %) 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, SC, ISA, mCP and OPSP were

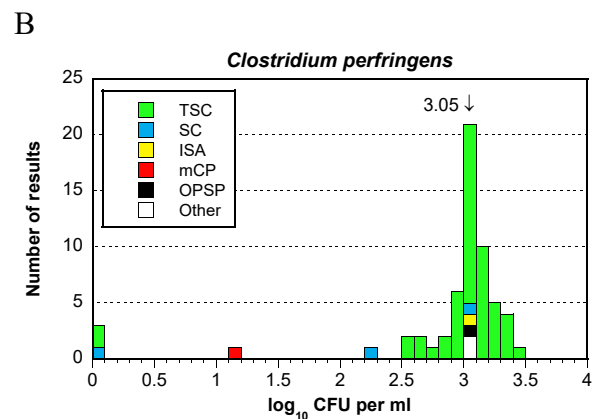
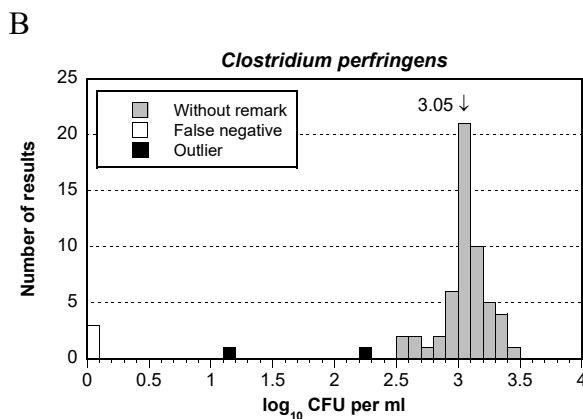
used by a few laboratories. Due to the low number of users, comparisons with TSC are difficult to make. It could here be mentioned two studies that recommend TSC for the analysis of *C. perfringens* in food samples (2, 3).

Two common methods for confirmation of *C. perfringens* are motility test and test for lactose fermentation; *C. perfringens* is non-motile and forms acid and gas as a consequence of lactose fermentation. *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.

C. perfringens normally grows both at 37 °C and at 44 °C. Here, the majority of the laboratories (93 %) incubated at 37 °C, while only a few (7 %) 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	58	58	-	-	0	-	-	59	54	3.05	0.19	3	2	0	59	48	-	-	11	-	-
TSC	50	50	-	-	0	-	-	51	49	3.05	0.20	2	0	0	51	41	-	-	10	-	-
SC	3	3	-	-	0	-	-	3	1	-	-	1	1	0	3	2	-	-	1	-	-
ISA	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
mCP	1	1	-	-	0	-	-	1	0	-	-	0	1	0	1	1	-	-	0	-	-
OPSP	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
Other	2	2	-	-	0	-	-	2	2	-	-	0	0	0	2	2	-	-	0	-	-



Anaerobic sulphite-reducing bacteria

Sample A

No target organism was present in the sample.

One false positive result was reported.

Sample B

The strain of *C. perfringens* was target organism. On ISA, it forms black colonies. The black colour may be somewhat less distinct after 48 hours, compared to after 24 hours. Only checking the plates after 48 hours may therefore give low results.

One high outlier was reported, as well as one false negative result.

Sample C

The strain of *C. bifermentans* was target organism. It forms black colonies on ISA.

Two low and one high outliers were reported, as well as three false negative results. The results for PAB were lower compared to other media.

General remarks

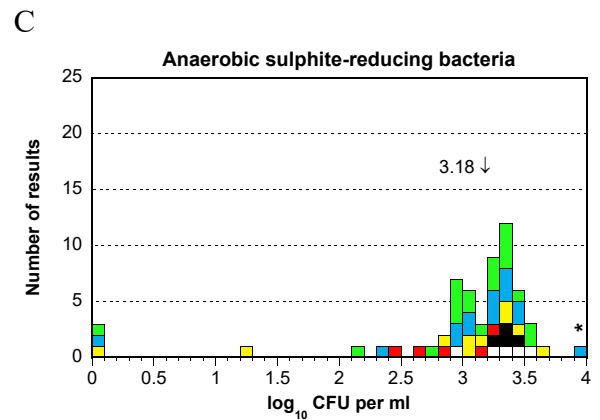
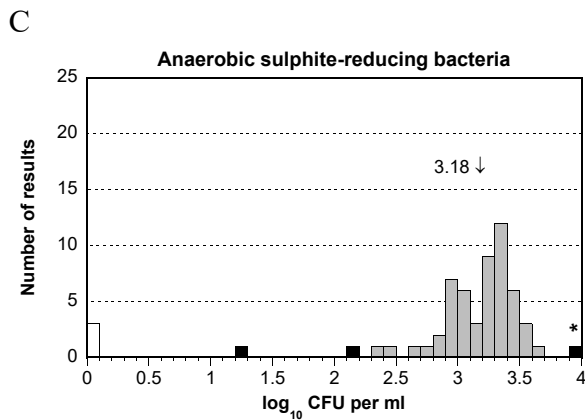
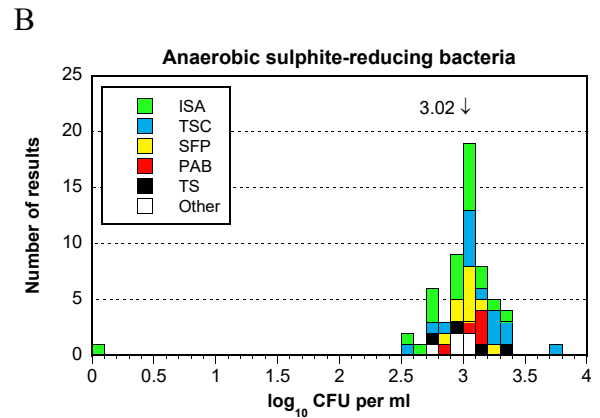
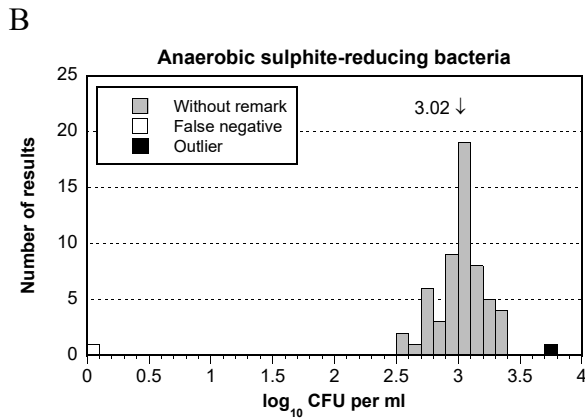
As in previous proficiency testing rounds, the majority of the laboratories followed a version of NMKL 56. The proportion of users of NMKL 56:2015 was somewhat higher than previously, and it was now used by 20 % of the laboratories. Most laboratories however still followed either NMKL 56:2008 (44 %) or the considerably older NMKL 56:1994 (3 %). 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. One laboratory 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 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 Fe^{3+} in the medium, and H_2S that is produced by the reduction of sulphite. Growth of anaerobic bacteria that only produce hydrogen (and not H_2S) 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. Laboratories that incubated on PAB did report comparably lower results for sample C, but since the number of users is low, it cannot be ruled out that this was simply due to chance.

Results from analysis of anaerobic sulphite-reducing 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	61	60	-	-	1	-	-	59	57	3.02	0.18	1	0	1	59	53	3.18	0.26	3	2	1
ISA	22	22	-	-	0	-	-	20	19	2.97	0.20	1	0	0	20	18	3.17	0.21	1	1	0
TSC	15	14	-	-	1	-	-	15	14	3.08	0.22	0	0	1	15	13	3.17	0.30	1	0	1
SFP	10	10	-	-	0	-	-	10	10	3.02	0.11	0	0	0	10	8	3.22	0.26	1	1	0
PAB	5	5	-	-	0	-	-	5	5	3.06	0.12	0	0	0	5	5	2.86	0.32	0	0	0
TS	4	4	-	-	0	-	-	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Other	5	5	-	-	0	-	-	5	5	-	-	0	0	0	5	5	-	-	0	0	0



Aerobic microorganisms in fish products, 20–25 °C

Sample A

The strains of *B. cereus*, *L. plantarum* and *E. coli* were present in the highest concentrations and were thus the main target organisms.

No outliers or false negative results were reported.

Sample B

The strains of *S. aureus* and *A. caviae* were present in the highest concentrations and were thus the main target organisms.

One low outlier was reported.

Sample C

The strains of *E. coli*, *L. plantarum* and *A. hydrophila* were present in the highest concentrations and were thus the main target organisms.

One low outlier was reported.

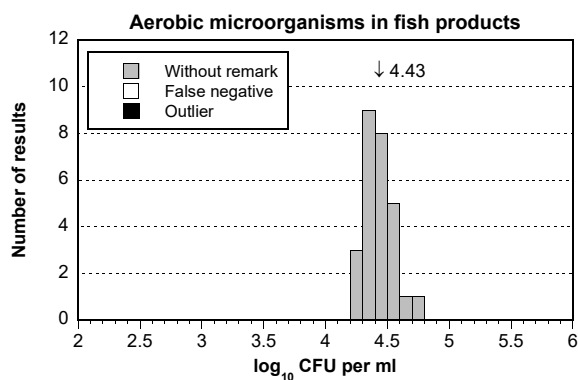
General remarks

The majority of the laboratories (86 %) 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 (likely) 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.

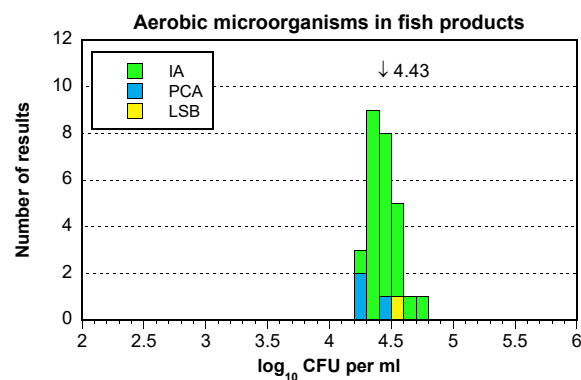
Results from analysis of aerobic microorganisms in fish products, 20–25 °C

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	27	27	4.43	0.13	0	0	0	28	27	4.02	0.10	0	1	0	28	27	4.48	0.16	0	1	0
IA	23	23	4.44	0.12	0	0	0	24	23	4.02	0.09	0	1	0	24	23	4.47	0.17	0	1	0
PCA	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
LSB	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

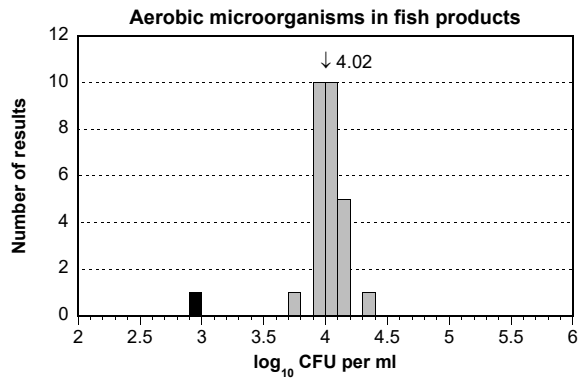
A



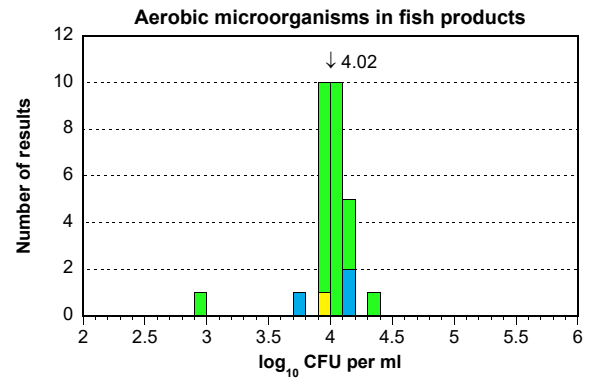
A



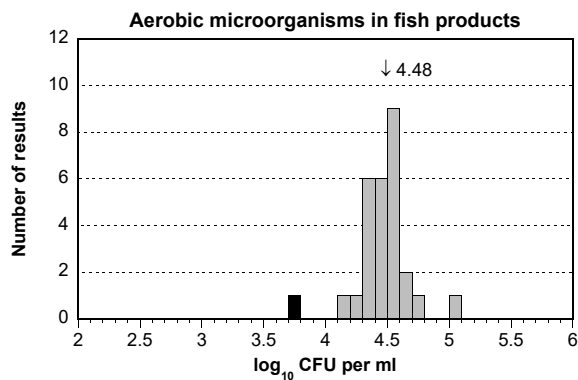
B



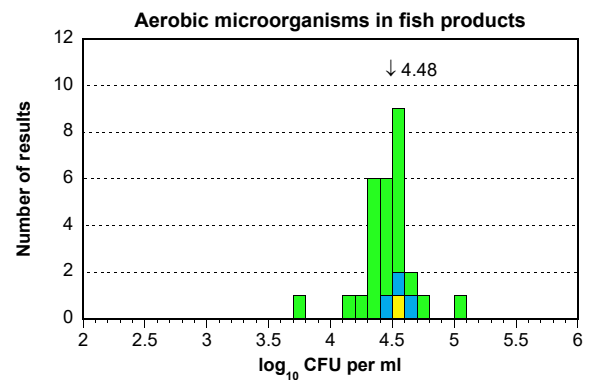
B



C



C



H₂S-producing bacteria in fish products

Sample A

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

One false positive result was reported.

Sample B

No target organism was present in the sample.

Two false positive results were reported.

Sample C

The strain of *H. alvei* was target organism. It forms black colonies on IA.

One high outlier was reported, as well as two false negative results.

General remarks

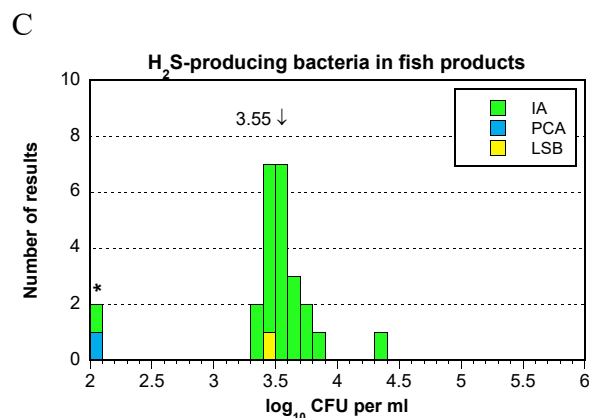
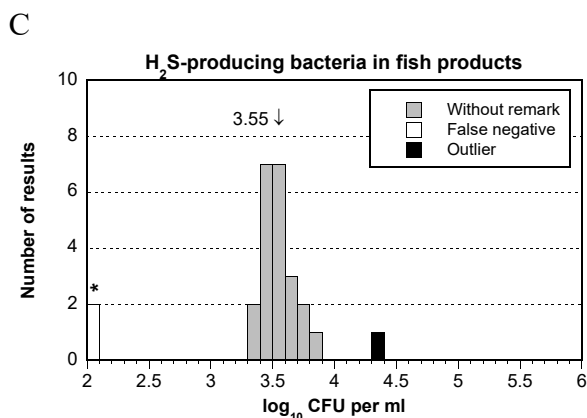
The majority of the laboratories (92 %) followed the method for aerobic microorganisms and specific spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with IA, on which H₂S-producing bacteria form black colonies. One laboratory followed NMKL 96:2003 ("Bacterial examinations in fresh and frozen seafood"), which includes the analysis of H₂S-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.

One laboratory followed ISO 4833-1:2013 and thus incubated on PCA. It is unclear how this laboratory would identify H₂S-producing bacteria on this medium. The laboratory reported zero results for all three samples.

Results from analysis of H₂S-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	24	23	-	-	1	-	-	25	23	-	-	2	-	-	25	22	3.55	0.12	2	0	1
IA	22	21	-	-	1	-	-	23	21	-	-	2	-	-	23	21	3.55	0.12	1	0	1
PCA	1	1	-	-	0	-	-	1	1	-	-	0	-	-	1	0	-	-	1	0	0
LSB	1	1	-	-	0	-	-	1	1	-	-	0	-	-	1	1	-	-	0	0	0



Yeasts and moulds

Sample A

The strain of *K. marxianus* was target organism for the analysis of yeasts. Three low and four high outliers were reported, as well as two false negative results.

The strain of *C. cladosporioides* was target organism for the analysis of moulds. Three low and one high outliers were reported, as well as ten false negative results.

Five of the 129 laboratories analysed with TEMPO YM, which gives a combined result for yeasts and moulds. Since the number of results for TEMPO YM is too low to allow a statistical analysis, they are instead evaluated based on the expected result for yeasts + moulds, and on the previous performance of the method in the Swedish Food Agency's proficiency testing. According to the Swedish Food Agency's quality control, the combined concentration of yeasts and moulds is $m_{SLV} = 2.55 \log_{10} \text{cfu ml}^{-1}$. Analyses with TEMPO YM have during 2016–2020 obtained a pooled standard deviation (s_{TEMPO}) of 0.34. **For TEMPO YM, results between $m_{SLV} \pm 2 s_{TEMPO}$ are considered acceptable, which corresponds to results between 1.87 and 3.24 $\log_{10} \text{cfu ml}^{-1}$.**

Sample B

The strain of *C. glabrata* was target organism for the analysis of yeasts. Six low and two high outliers were reported, as well as two false negative results.

The strain of *P. verrucosum* was target organism for the analysis of moulds. Four high outliers were reported, as well as 31 false negative results. There is no single explanation to the many false negative results. The strain of *P. verrucosum* was present in approximately $3.2 \log_{10} \text{ cfu ml}^{-1}$ in the sample and the analysis was without problem at the Swedish Food Agency (incubation on DG18 and DRBC at 25 °C for 7 days). The strain is also included in the Swedish Food Agency's reference material RM Food 2019:7. Only one of the false negative results appears to be due to mistaking mould colonies for yeasts. Eight of the false negative results were reported by laboratories that obtained a zero result also for sample A, meaning they may have had general problems with analysing moulds. Five laboratories appear to have had various problems, e.g. analysing the wrong sample. The remaining 17 false negative results are from laboratories that otherwise reported correct results for both yeasts and moulds. These 17 laboratories almost exclusively used either YGC (9 laboratories) or Petrifilm YM/RYM (4 laboratories). Also in previous PT rounds, false negative results have been reported for this particular strain when using YGC and Petrifilm YM/RYM, and to a similar extent as in the present PT.

Five of the 129 laboratories analysed with TEMPO YM. **Following the same principle as for sample A, results for TEMPO YM between 2.55 and 3.92 $\log_{10} \text{ cfu ml}^{-1}$ are considered acceptable.**

Sample C

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

Six false positive results were reported for yeasts, and two false positive results for moulds.

General remarks

In principle, the laboratories analysed both 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. ISO 21527-1:2008 uses DRBC while ISO 21527-2:2008 used DG18. In general, DRBC is recommended for food with water activity $a_w > 0.95$ (e.g. fresh 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).

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.

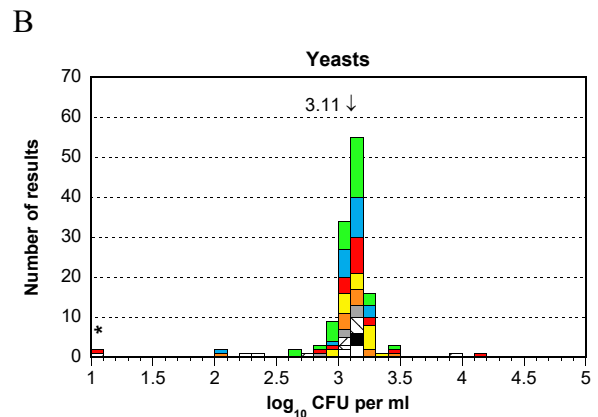
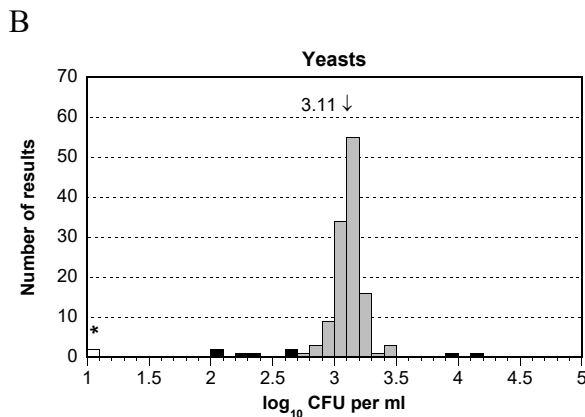
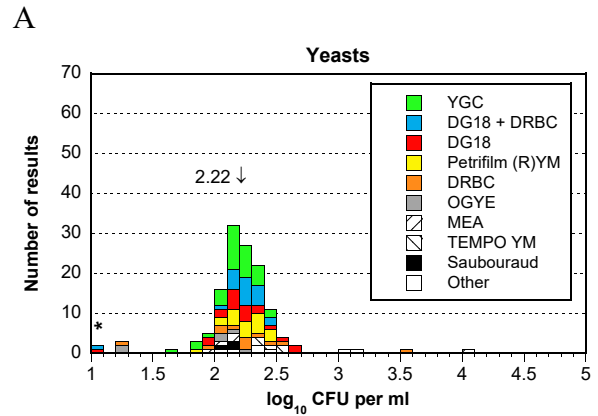
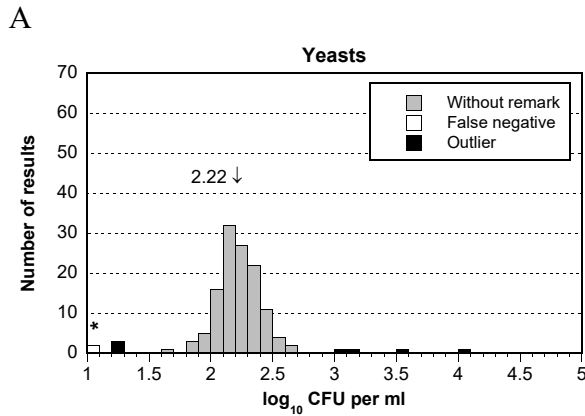
As discussed above, five laboratories used TEMPO YM, sometimes in combination with other methods/media. The results from these five laboratories have been excluded when determining outliers, but they are otherwise for practical reasons included in

tables and figures in this report. There, they may (inaccurately) appear as outliers or false results. **Results from TEMPO YM are however specifically—and only—evaluated according to the limits provided above for the respective samples.**

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	132	123	2.22	0.17	2	3 4	132	122	3.11	0.11	2	6 2	133	127	-	-	6	-	-
YGC	34	34	2.17	0.17	0	0 0	34	32	3.11	0.11	0	2 0	35	33	-	-	2	-	-
DG18 + DRBC	21	20	2.26	0.11	1	0 0	22	21	3.11	0.08	0	1 0	21	20	-	-	1	-	-
DG18	20	19	2.24	0.21	1	0 0	20	18	3.12	0.12	1	0 1	20	19	-	-	1	-	-
Petrifilm YM/RYM	19	19	2.24	0.17	0	0 0	18	18	3.13	0.11	0	0 0	19	19	-	-	0	-	-
DRBC	12	10	2.22	0.19	0	1 1	12	11	3.16	0.13	0	1 0	12	12	-	-	0	-	-
OGYE	6	4	2.12	0.09	0	2 0	6	6	3.05	0.11	0	0 0	6	6	-	-	0	-	-
MEA	5	4	-	-	0	0 1	5	3	-	-	1	0 1	5	3	-	-	2	-	-
TEMPO YM	5	5	-	-	0	0 0	5	5	-	-	0	0 0	5	5	-	-	0	-	-
Saubouraud	3	3	-	-	0	0 0	3	3	-	-	0	0 0	3	3	-	-	0	-	-
Other*	7	5	-	-	0	0 2	7	5	-	-	0	2 0	7	7	-	-	0	-	-

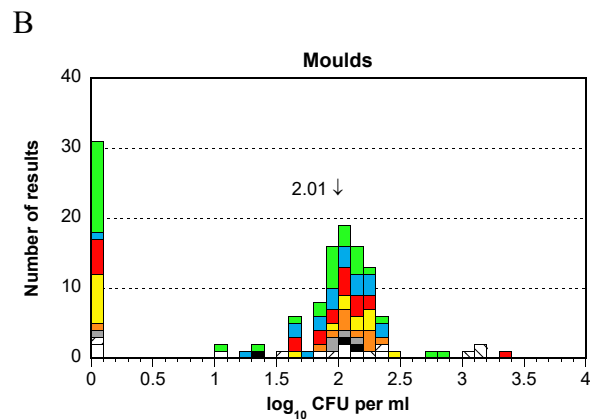
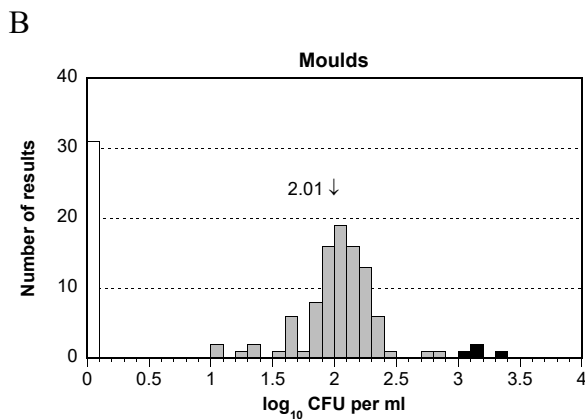
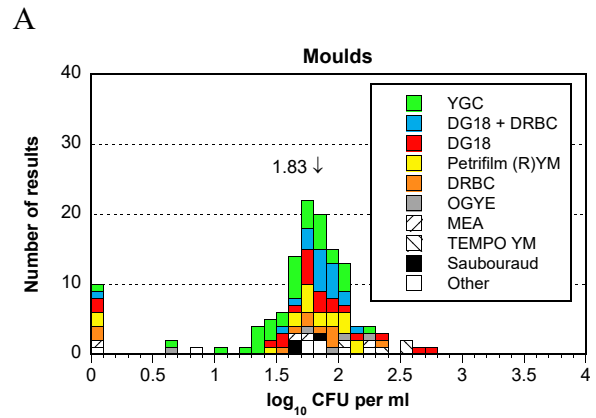
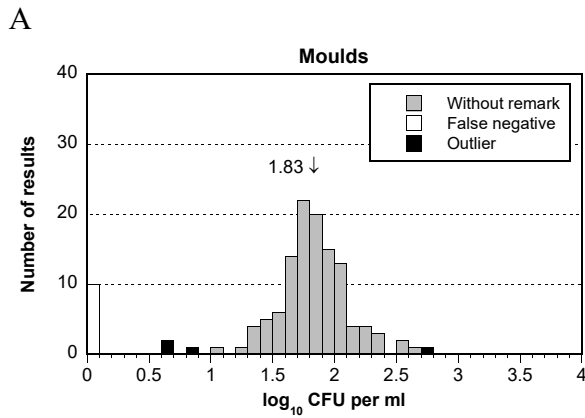
* Includes Compact Dry YM and PDA.



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	129	115	1.83	0.26	10	3	1	129	94	2.01	0.29	31	0	4	130	128	-	-	2	-	-
YGC	35	33	1.68	0.28	1	1	0	35	22	2.01	0.38	13	0	0	36	36	-	-	0	-	-
DG18 + DRBC	20	19	1.86	0.14	1	0	0	21	20	1.98	0.28	1	0	0	20	19	-	-	1	-	-
DG18	21	18	1.86	0.28	2	0	1	21	15	2.00	0.20	5	0	1	21	21	-	-	0	-	-
Petrifilm YM/RYM	18	16	1.85	0.19	2	0	0	17	10	2.11	0.24	7	0	0	18	17	-	-	1	-	-
DRBC	11	9	1.86	0.21	2	0	0	11	10	2.11	0.15	1	0	0	11	11	-	-	0	-	-
OGYE	5	4	-	-	0	1	0	5	4	-	-	1	0	0	5	5	-	-	0	-	-
MEA	4	3	-	-	1	0	0	4	3	-	-	1	0	0	4	4	-	-	0	-	-
TEMPO YM	4	4	-	-	0	0	0	4	1	-	-	0	0	3	4	4	-	-	0	-	-
Saubouraud	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	-	-
Other*	8	6	-	-	1	1	0	8	6	-	-	2	0	0	8	8	-	-	0	-	-

* Includes Compact Dry YM and PDA.



Outcome of the results of individual laboratory - assessment

Reporting and evaluation of results

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

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

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

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

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol (4). Samples for follow-up can be ordered, free of charge via our website: www.livsmedelsverket.se/en/PT-extra

Z-scores, box plots and deviating results

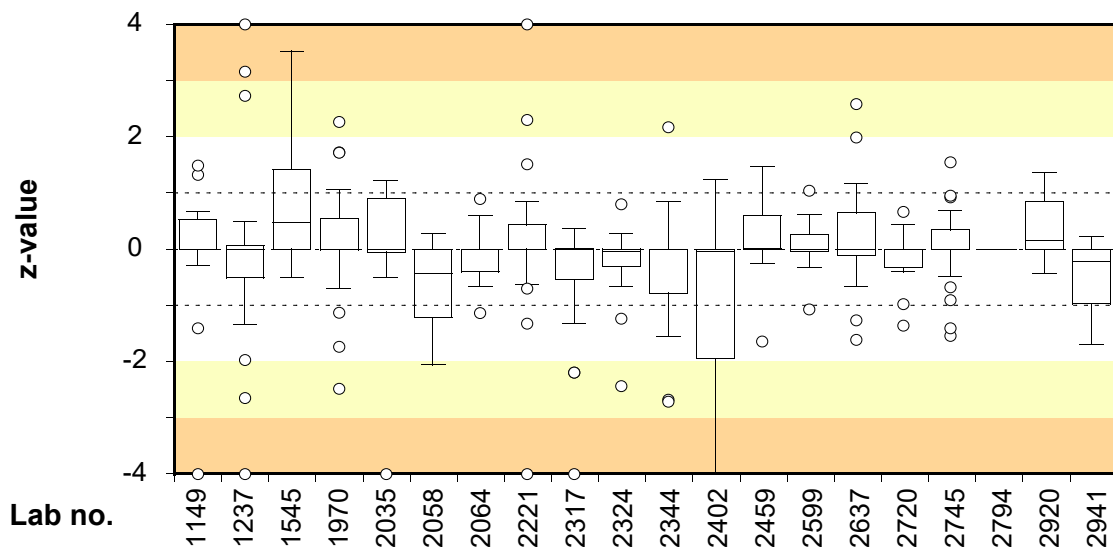
In order to allow comparison of the results from different analyses and mixtures, all results are transformed into standard values (z-scores). For quantitative analyses, a z-score is either positive or negative, depending on whether the individual result is higher or lower than the mean value calculated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

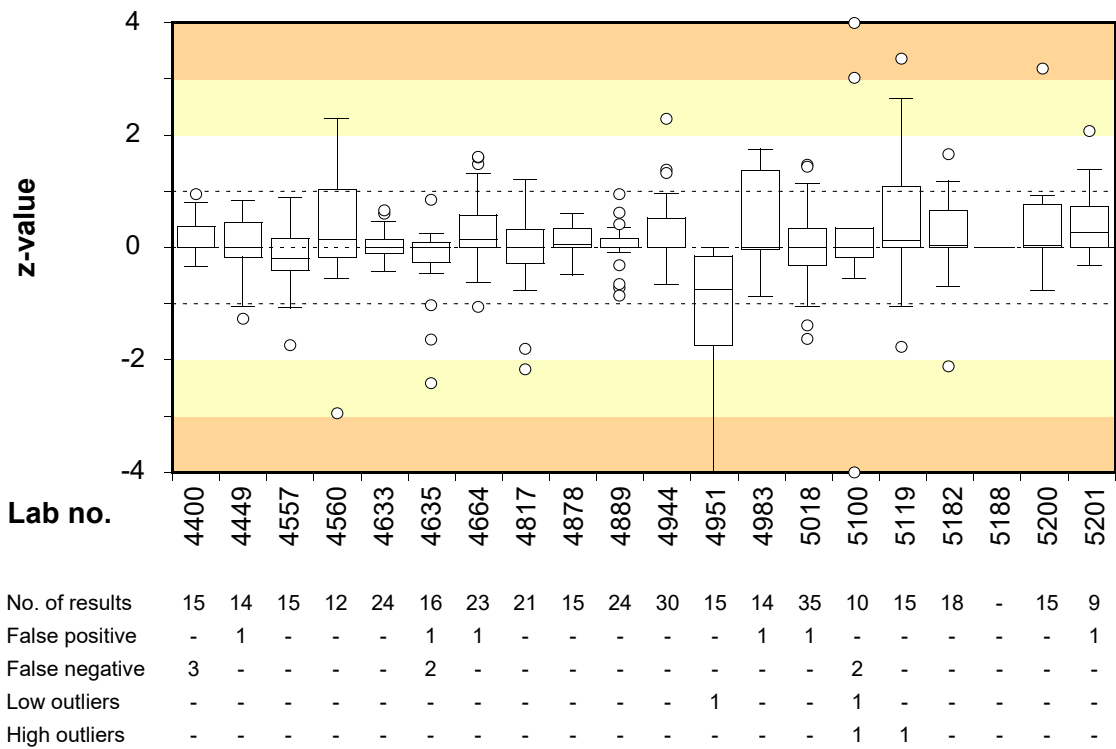
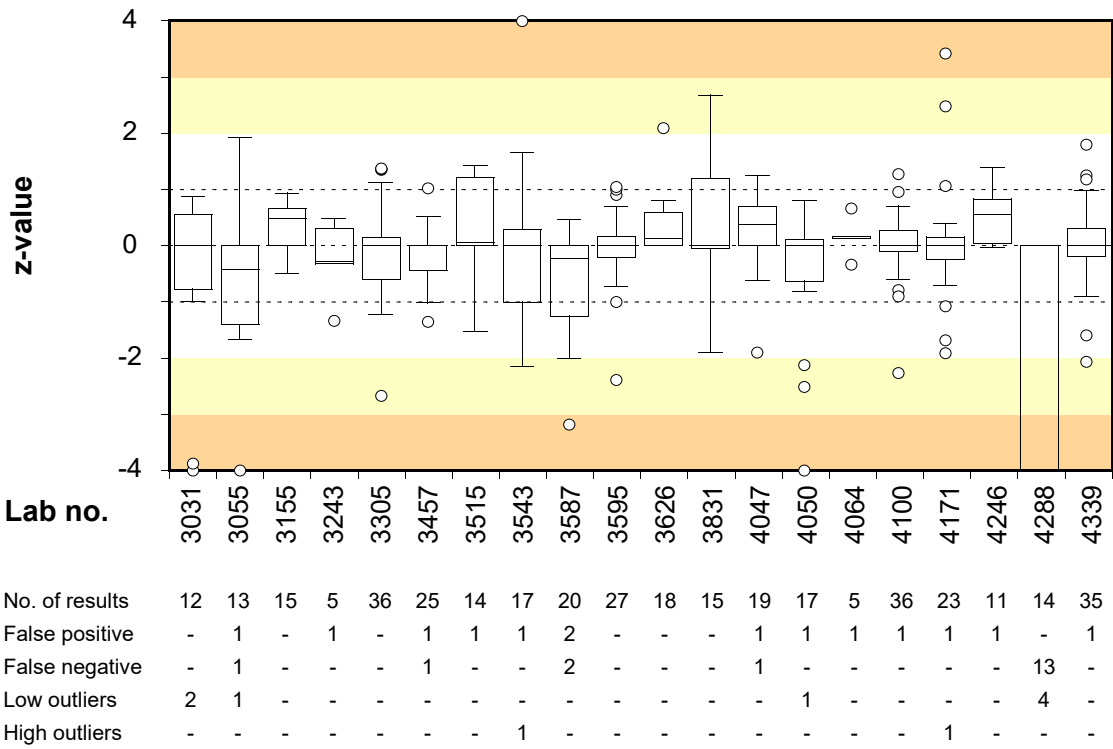
Box plots and numbers of deviating results for each laboratory

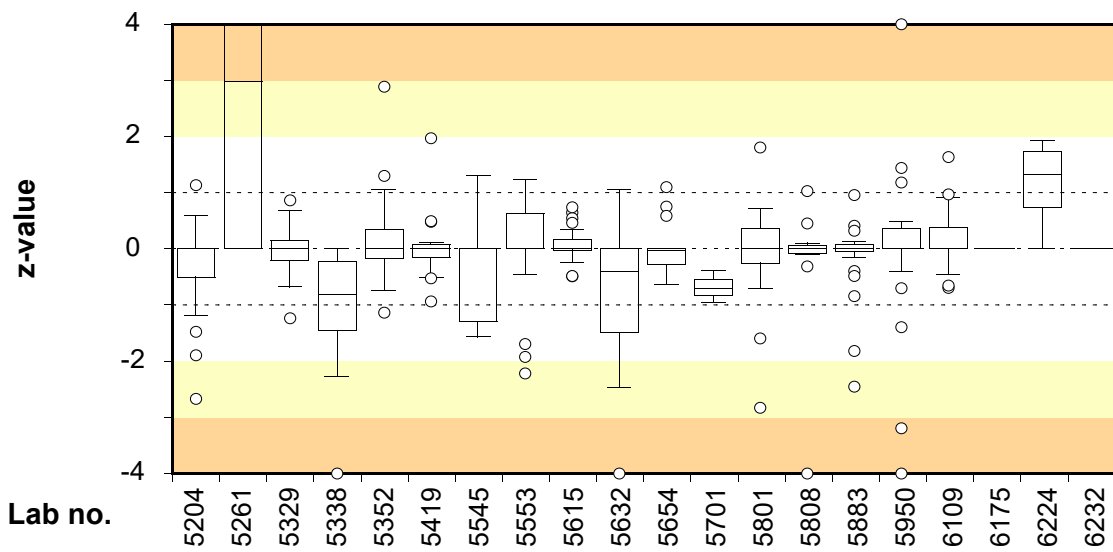
- Z-scores are calculated according to the formula: $z = (x-m)/s$, where x is the result of the individual laboratory, m is the mean of the results of all participating laboratories, and s is the standard deviation of the participating laboratories, after removing outliers and false results.
- Outliers 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.

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

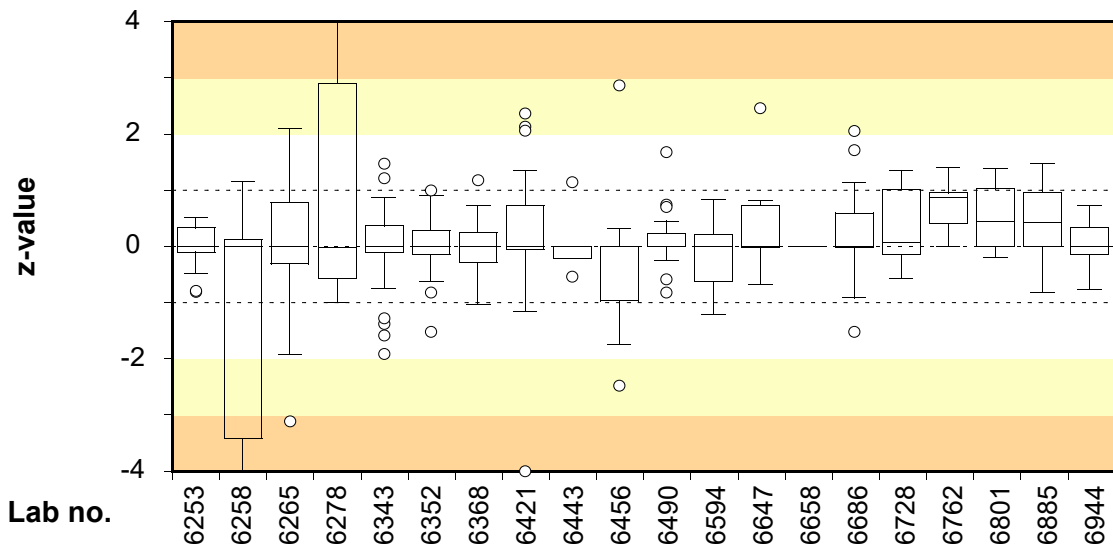


Lab no.	1149	1237	1545	1970	2035	2058	2064	2221	2317	2324	2344	2402	2459	2599	2637	2720	2745	2794	2920	2941
No. of results	18	28	29	36	12	20	15	30	26	22	28	15	20	18	28	13	27	-	12	23
False positive	-	6	1	1	-	1	-	-	1	-	-	-	1	-	1	1	-	-	-	1
False negative	-	3	-	-	-	-	-	-	-	-	1	-	-	-	1	1	-	-	-	1
Low outliers	1	2	-	-	1	-	-	1	1	-	-	3	-	-	-	-	-	-	-	-
High outliers	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-

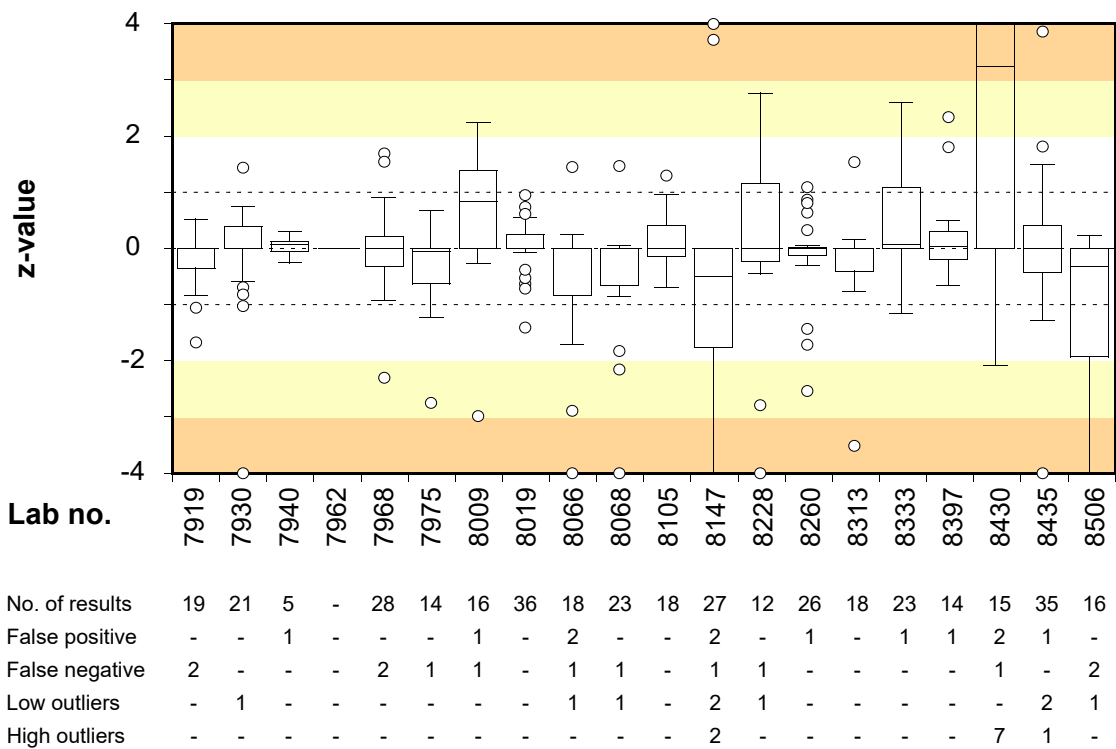
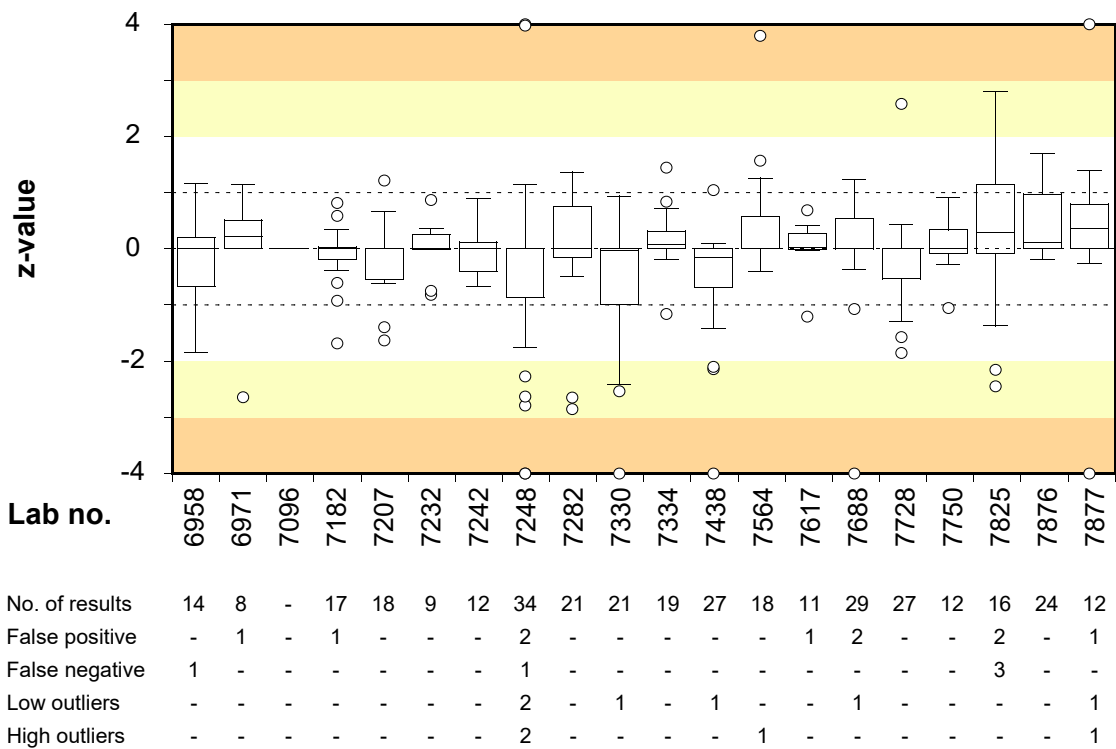


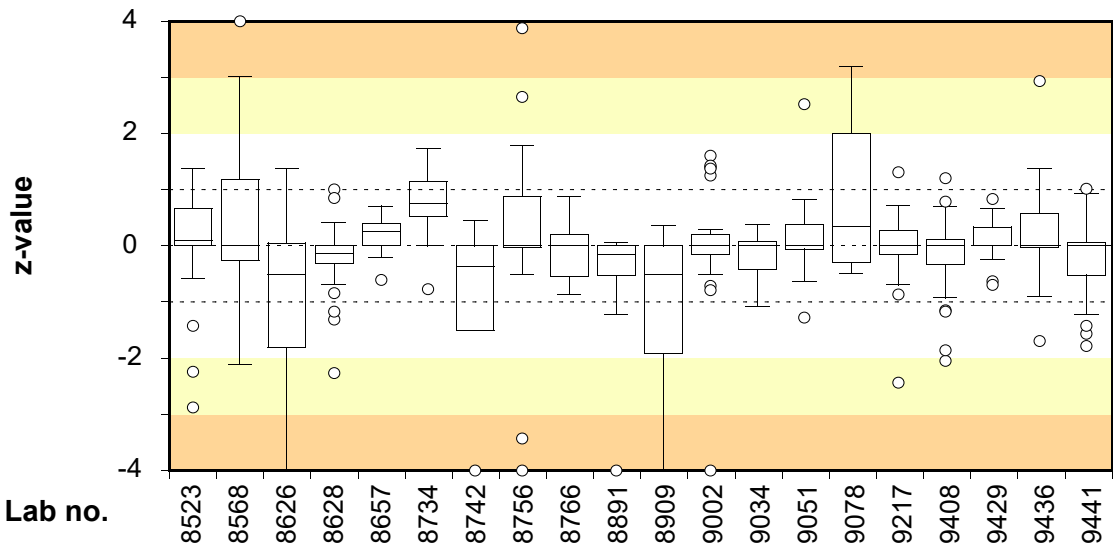


Lab no.	5204	5261	5329	5338	5352	5419	5545	5553	5615	5632	5654	5701	5801	5808	5883	5950	6109	6175	6224	6232
No. of results	30	13	20	11	24	16	14	18	25	15	14	3	14	12	25	29	21	-	6	-
False positive	1	1	1	1	-	-	-	-	1	-	1	-	-	-	2	4	-	-	2	-
False negative	-	1	3	-	-	-	1	-	1	-	-	-	1	3	-	4	-	-	1	-
Low outliers	-	-	-	1	-	-	-	-	-	1	-	-	-	1	-	2	-	-	-	-
High outliers	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-

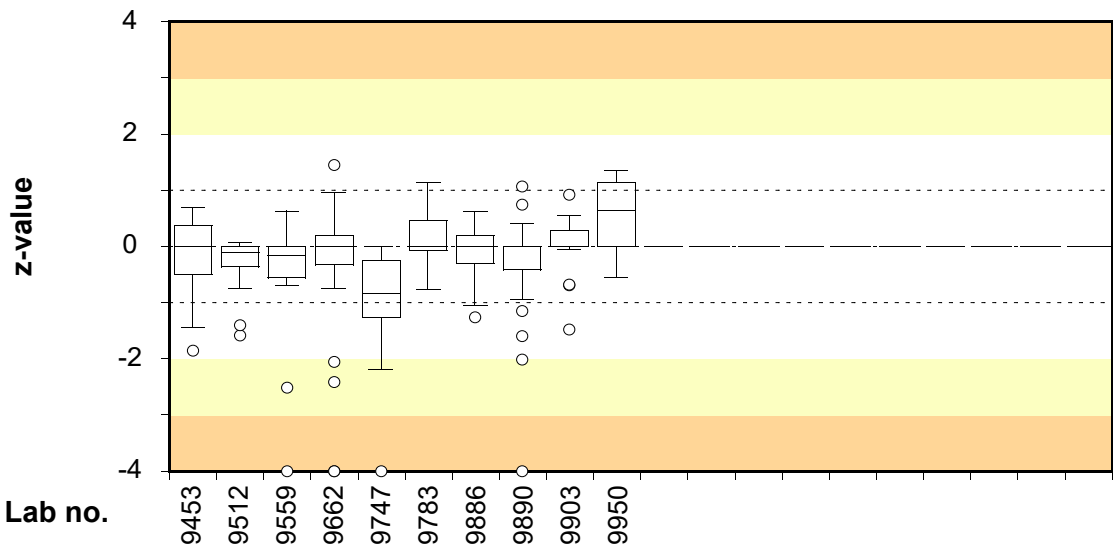


Lab no.	6253	6258	6265	6278	6343	6352	6368	6421	6443	6456	6490	6594	6647	6658	6686	6728	6762	6801	6885	6944
No. of results	18	9	25	8	27	27	33	33	6	21	21	22	9	-	24	12	9	12	24	15
False positive	-	-	1	1	3	-	-	2	-	-	-	2	1	-	1	-	-	1	-	-
False negative	-	1	2	-	-	-	-	1	-	-	-	-	-	-	3	-	-	2	-	-
Low outliers	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-





No. of results	17	22	12	35	12	10	9	20	24	16	17	27	10	9	4	14	34	21	31	31
False positive	1	2	-	1	-	2	-	1	-	1	1	1	-	-	1	1	2	-	-	-
False negative	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Low outliers	-	-	1	-	-	-	2	1	-	1	1	2	-	-	-	-	-	-	-	-
High outliers	-	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-



No. of results	17	16	21	32	11	11	29	24	24	14
False positive	-	-	-	3	2	-	2	-	-	1
False negative	1	2	-	1	2	1	-	-	-	-
Low outliers	-	-	1	2	1	-	-	1	-	-
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 (5). 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 ¹	Microorganism	Strain	
		SLV no. ²	Reference ³
A	<i>Bacillus cereus</i>	SLV-160	CCUG 45098
	<i>Lactobacillus plantarum</i>	SLV-475	CCUG 30503
	<i>Escherichia coli</i>	SLV-524	CCUG 47554
	<i>Staphylococcus xylosus</i>	SLV-283	Cheese, 1989
	<i>Kluyveromyces marxianus</i>	SLV-439	CBS G99-106
	<i>Cladosporium cladosporioides</i>	SLV-488	CBS 812.96
B	<i>Staphylococcus aureus</i>	SLV-280	isolated from egg
	<i>Clostridium perfringens</i>	SLV-442	CCUG 43593
	<i>Candida glabrata</i>	SLV-052	-
	<i>Penicillium verrucosum</i>	SLV-544	CBS 112488
	<i>Aeromonas caviae</i>	SLV-206	CCUG 43595
C	<i>Escherichia coli</i>	SLV-082	CCUG 45097
	<i>Staphylococcus aureus</i>	SLV-350	CCUG 45099
	<i>Lactobacillus plantarum</i>	SLV-445	ATCC 8014
	<i>Hafnia alvei</i>	SLV-015	CCUG 45642
	<i>Aeromonas hydrophila</i>	SLV-467	CCUG 46535
	<i>Clostridium bifermentans</i>	SLV-009	CCUG 43592

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

² Internal strain identification no. at the Swedish Food Agency

³ 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 6 and 7 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 ¹			B ²			C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.60	1.50	1.48	4.11	1.98	1.27	4.66	0.58	1.25
Psychrotrophic microorganisms NMKL method no. 86:2013	3.91	5.66	5.18	2.76	8.20	2.81	3.67	0.93	1.33
Enterobacteriaceae NMKL method no. 144:2005	3.65	1.76	1.48	-	-	-	4.23	1.62	1.33
<i>Escherichia coli</i> NMKL method no. 125:2005	3.70	2.13	1.54	-	-	-	4.17	1.50	1.33
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	4.51	2.36	1.69	-	-	-	-	-	-
Coagulase-positive staphylococci NMKL method no. 66:2009	2.98 ³	0.97 ³	2.08 ³	4.00	0.46	1.14	3.51	0.30	1.21
Lactic acid bacteria NMKL method no. 140:2007	3.70	2.35	1.52	-	-	-	4.27	0.24	1.11
<i>Clostridium perfringens</i> NMKL method no. 95:2009	-	-	-	3.04	2.03	1.29	-	-	-
Anaerobic sulphite-reducing bacteria NMKL method no. 56:2015	-	-	-	3.04	0.47	1.14	3.18	0.61	1.19
Aerobic microorganisms in fish products NMKL method no. 184:2006	4.72	3.16	1.67	4.10	1.58	1.26	4.65	2.78	1.58
H ₂ S-producing bacteria in fish products NMKL method no. 184:2006	-	-	-	-	-	-	3.81	0.73	1.89
Yeasts NMKL method no. 98:2005	2.39	0.76	1.42	3.18	1.77	1.25	-	-	-
Moulds NMKL method no. 98:2005	2.05	0.29	1.36	2.31	1.77	1.91	-	-	-

- No target organism and therefore no value

¹ n = 5 vials analysed in duplicate

² n = 10 vials analysed in duplicate

³ Not target organism for the analysis

References

1. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Anal. Chem.* 73:58–64.
2. de Jong A.E.I., Eijhusen, G.P., Brouwer-Post, E.J.F., Grand, M., Johansson, T., Kärkkäinen, T., Marugg, J., in't Veld, P.H., Warmerdam, F.H.M., Wörner, G., Zicavo, A., Rombouts, F.M., Beumer, R.R. 2003. Comparison of media for enumeration of *Clostridium perfringens* from foods, *Journal of Microbiological Methods*, 54(3):359–366.
3. Byrne, B., Scannell, A.G.M., Lyng, J., Bolton, D.J. 2008. An evaluation of *Clostridium perfringens* media, *Food Control* 19(11):1091–1095
4. Anonymous, 2018. Protocol. Microbiology. Drinking water & Food, Swedish Food Agency.
5. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
6. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.
7. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockfeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.

Lab no	Vial	Aerobic microorganisms 30 °C			Psychrotrophic microorganisms			Enterobacteriaceae			<i>Escherichia coli</i>			Presumptive <i>Bacillus cereus</i>			Coagulase-positive staphylococci			Lactic acid bacteria			<i>Clostridium perfringens</i>			Anaerobic sulphite-reducing bacteria			Aerobic m.o. in fish products, 20-25 °C			H ₂ S-prod. bacteria in fish products			Yeasts			Moulds			Lab no
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
9950	1 3 2	4.5	3.97	4.55	-	-	-	3.77	0	4.28	-	-	-	-	-	-	-	-	-	3.78	1.4	4.33	-	-	-	-	-	-	-	-	-	-	-	-	2.38	3.22	0	1.9	2.26	0	9950

N	147	149	148	20	21	21	128	128	130	113	116	114	113	115	115	97	99	99	56	55	56	58	59	59	61	59	59	27	28	28	24	25	25	132	132	133	129	129	130	N			
Min	1.30	2.26	2.38	0	0	1.00	0	0	0	0	0	0	0	0	0	0	0	0	3.08	0	3.15	0	0	0	0	0	0	4.20	2.93	3.76	0	0	0	0	0	0	0	0	0	0	0	0	Min
Max	5.23	5.06	5.22	4.55	4.07	4.75	4.66	3.40	4.45	4.75	2.38	5.02	5.36	3.75	4.63	4.12	5.48	4.41	4.31	4.00	4.70	0	3.48	3.40	3.82	3.77	4.16	4.72	4.30	5.00	3.78	3.00	4.34	4.08	4.17	4.41	2.72	3.32	2.23	Max			
Med	4.41	4.03	4.58	4.05	2.43	3.56	3.61	0	4.10	3.58	0	4.00	4.36	0	0	0	3.94	3.45	3.59	0	4.22	0	3.07	0	0	3.02	3.23	4.43	4.00	4.49	0	0	3.57	2.23	3.12	0	1.82	2.03	0	Med			
m	4.386	4.034	4.573	3.463	2.185	3.688	3.607	0	4.087	3.576	0	3.933	4.349	0	0	0	3.949	3.434	3.585	0	4.208	0	3.050	0	0	3.017	3.176	4.427	4.017	4.482	0	0	3.551	2.218	3.113	0	1.825	2.013	0	m			
s	0.257	0.118	0.134	1.362	1.302	0.407	0.140	0	0.170	0.188	0	0.236	0.189	0	0	0	0.097	0.112	0.144	0	0.105	0	0.187	0	0	0.180	0.265	0.127	0.104	0.164	0	0	0.123	0.170	0.107	0	0.261	0.293	0	s			
u_(lg)	0.021	0.010	0.011	0.305	0.284	0.091	0.013	0	0.015	0.019	0	0.023	0.019	0	0	0	0.010	0.012	0.020	0	0.015	0	0.025	0	0	0.024	0.036	0.024	0.020	0.032	0	0	0.026	0.015	0.010	0	0.024	0.030	0	u_(lg)			
F+	0	0	0	0	0	0	0	4	0	0	1	0	0	2	7	7	0	0	0	23	0	0	0	11	1	0	0	0	0	0	1	2	0	0	0	6	0	0	2	F+			
F-	0	0	0	0	0	0	2	0	2	7	0	2	7	0	0	0	4	5	0	0	0	0	3	0	0	1	3	0	0	0	0	0	2	2	2	0	10	31	0	F-			
<	3	4	3	0	0	1	3	0	2	2	0	2	6	0	0	0	2	4	1	0	4	0	2	0	0	0	2	0	1	1	0	0	0	3	6	0	3	0	0	<			
>	0	2	2	0	0	0	1	0	0	1	0	1	1	0	0	0	2	3	2	0	1	0	0	0	0	1	1	0	0	0	0	0	1	4	2	0	1	4	0	>			
< OK	3.63	3.62	4.20	0	0	3.00	3.26	0	3.54	3.04	0	3.31	3.70	0	0	0	3.67	3.10	3.20	0	3.96	0	2.51	0	0	2.53	2.37	4.20	3.76	4.14	0	0	3.34	1.63	2.78	0	1.00	1.04	0	< OK			
> OK	5.23	4.41	4.88	4.55	4.07	4.75	4.04	0	4.45	4.18	0	4.42	4.92	0	0	0	4.23	3.61	3.91	0	4.48	0	3.48	0	0	3.33	3.60	4.72	4.31	5.00	0	0	3.83	2.70	3.49	0	2.61	2.84	0	> OK			

N = number of analyses performed
Min = lowest reported result

Max = highest reported result
Med = median value

m = mean value
s = standard deviation

F+ = false positive
F- = false negative


< = low outlier
> = high outlier

< OK = lowest accepted value
> OK = highest accepted value

u_(lg) = measurement uncertainty for assigned value (m)

- 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 microorg.			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 ₂ S-prod. bacteria in fish products			Yeasts			Moulds			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
9886	1	3	2	-0.257	-0.626	0.204			-0.513	-1.050	0	-0.041	-1.257	0	-0.607	0.430	0	0	0	-0.300	0.408	0.032		0.588	0	0.321		0	-0.373	0.394							-0.224	0.631	0	-0.595	0.570	0	9886
9890	3	2	1	0.405	-4.000	-1.590				-0.120	0	-0.453	-0.246	0	0.115	0.747	0	0	0	-0.094	-0.306	-0.383	0	-0.940													1.072	-1.145	0	-2.012	0.092	0	9890
9903	3	2	1	-0.685	0.392	0.129				-0.049	0	0.313	0.552	0	0.922	-1.473	0	0	0	0.318	0.229																0.070	-0.678	0	0.439	0.229	0	9903
9950	1	3	2	0.444	-0.541	-0.170				1.167	0	1.137										1.348		1.162													0.954	1.004	0	0.285	0.843	0	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