

Proficiency testing Food Microbiology

April 2022

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Abbreviations

Media

ALOA	Agar for <i>Listeria</i> according to Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BA	Blood agar
BcsA	<i>Bacillus cereus</i> selective agar
BEA	Bile esculin agar
BGA	Brilliant green agar
BGLB	Brilliant green lactose bile broth
BP	Baird-Parker agar
BPW	Buffered peptone water
BS	Bromthymol blue saccharose agar
CBC	Oxoid Brilliance™ <i>Bacillus cereus</i> agar
CIN	Cefsulodin irgasan novobiocin agar
Compact Dry EC	Compact Dry™ <i>E. coli</i> and coliforms
Compact Dry ETB	Compact Dry™ Enterobacteriaceae
Compact Dry ETC	Compact Dry™ Enterococcus
Compact Dry TC	Compact Dry™ Total Count
COMPASS	COMPASS® Enterococcus agar
CT-SMAC	Cefixime tellurite sorbitol MacConkey agar
DG18	Dikloran glycerol agar
DRBC	Dikloran Rose-Bengal chloramphenicol agar
EC	<i>E. coli</i> broth
ENT	Slanetz & Bartley <i>Enterococcus</i> agar
HEA	Hektoen enteric agar
IA	Iron agar
ISA	Iron sulphite agar
ITC	Irgasan ticarcillin potassium chlorate broth
KEAA	Kanamycin esculin azide agar
LMBA	<i>Listeria monocytogenes</i> blood agar
LSB	Lauryl sulphate broth
LTLSB	Lactose tryptone lauryl sulphate broth
mCCDA	Modified charcoal cephaloperazone deoxycholate agar
mCP	Membrane <i>Clostridium perfringens</i> agar
MKTTn	Muller-Kauffmann tetrathionate/novobiocin broth
MPCA	Milk plate count agar
MRB	Modified Rappaport broth
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
MSRV	Modified semi-solid Rappaport-Vassiliadis enrichment media
mTSB	Modified tryptone soya broth

MYP	Mannitol egg yolk polymyxin agar
OCLA	Oxoid Brilliance™ Listeria agar
OGYE	Oxytetracyclin glucose yeast extract agar
OPSP	Oleandomycin, Polymixin, Sulphadiazine, Perfringens agar
PAB	Perfringens agar base
PDA	Potato dextrose agar
PALCAM	Polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar
Petrifilm AC	3M™ Petrifilm™ Aerobic Count
Petrifilm CC	3M™ Petrifilm™ Coliform 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 RAC	3M™ Petrifilm™ Rapid Aerobic Count
Petrifilm REC	3M™ Petrifilm™ Rapid <i>E. coli</i> /Coliform count
Petrifilm SEC	3M™ Petrifilm™ Select <i>E. coli</i>
Petrifilm Staph	3M™ Petrifilm™ Staph Express
PEMBA	Polymyxin pyruvate egg yolk mannitol bromothymol blue agar
PSB	Peptone sorbitol bile salts broth
PCA	Plate count agar
RPFA	Baird-Parker agar with rabbit plasma fibrinogen
SFA	Sugar-free agar
RVS	Rappaport-Vassiliadis Soy peptone broth
Saubouraud	Saubouraud chloramphenicol agar
SC	Sulphite cycloserine agar
SFP	Shahidi-Ferguson Perfringens agar
SMAC	Sorbitol MacConkey agar
SP	Salt Polymyxin broth
SSDC	Salmonella/Shigella sodium deoxycholate calcium chloride agar
TBX	Tryptone bile X-glucuronide agar
TCBS	Thiosulphate citrate bile salts sucrose agar
TGE	Tryptone glucose extract agar
TEMPO AC	TEMPO® Aerobic count
TEMPO BC	TEMPO® <i>Bacillus cereus</i>
TEMPO CAM	TEMPO® Campylobacter
TEMPO CC	TEMPO® Coliform count
TEMPO EB	TEMPO® Enterobacteriaceae
TEMPO EC	TEMPO® <i>E. coli</i>
TEMPO RYM	TEMPO® Rapid Yeast/Mould
TEMPO STA	TEMPO® Coagulase-positive staphylococci
TEMPO YM	TEMPO® Yeast/Mould
TGE	Tryptone glucose extract agar
TS	Tryptose sulphite agar
TSA	Tryptic soya agar
TSC	Tryptose sulphite cycloserine agar

TSBY	Tryptone soya broth with yeast extract
XLD	Xylose lysine deoxycholate 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
NordVal	NordVal International - NMKL
SLV	Livsmedelsverket/Swedish Food Agency, Sweden

Analyses in this PT round

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

Method

Reporting of results and method information

It is the responsibility of the individual participants to correctly report results according to the instructions. Incorrectly reported results, for example results reported for the wrong sample, cannot be correctly processed. Incorrectly reported results are as a general rule excluded but may – after manual assessment by the Swedish Food Agency in each individual case – still be included and processed.

According to EN ISO/IEC 17043, for which the proficiency testing (PT) programme is accredited, it is mandatory for the participants to report method information for all analyses. This method information is sometimes contradictory or difficult to interpret. For example when participants state a medium that is not included in the standard method they refer to, or when manual comments by the participant contradict the reported method information. In such cases, the reported method information provided by the participants is generally used in method comparisons “as it is”. Alternatively, method data that are difficult to interpret may be excluded or added to the group “Other”, together with results from methods and media that are only used by 1–2 participants.

Outliers

For analyses where more than 20 participants 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 participants have reported results, as well as in some individual cases, subjective adjustments are made to set outlier limits based on prior knowledge of the samples contents.

Assigned value and measurement uncertainty

Results reported by participants as “> value” are not evaluated. Results reported as “< value” are treated as zero (negative result).

Normally, mean values and standard deviations are provided for all analytical parameters with quantitative results. 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.

The assigned value (m_{PT}) is the arithmetic mean value of the participants’ results. The standard uncertainty (u_{PT}) of the assigned value is calculated as the standard deviation (s_{PT}) divided by the square root of the number of correct results (n):

$$u_{PT} = \frac{s_{PT}}{\sqrt{n}}$$

Z-scores

To allow comparison of the results from different analyses and samples, results are transformed into standard values (z-scores). Z-scores are calculated as:

$$z = \frac{x_{lab} - m_{PT}}{s_{PT}}$$

where x_{lab} is the result of the individual participant.

Z-scores for individual analyses are shown in Annex 2 and can be used as a tool by participants when following up on the results. For quantitative analyses, a z-score is either positive or negative, depending on whether the participants result is higher or lower than m_{PT} .

In evaluations of the analytical results, the following guidelines can be used:

- $|z| \leq 2$ indicates that the result is acceptable
- $2 < |z| < 3$ indicates a warning that the result may be deviating, and might motivate an action in the follow-up process
- $|z| \geq 3$ indicates that the result is regarded as deviating and should lead to an action in the follow-up process

Table legends





- N number of participants that reported results for the analysis
- n number of participants with satisfactory result (false results and outliers excluded)
- m_{PT} assigned value, mean value in \log_{10} cfu ml^{-1} (false results and outliers excluded)
- s_{PT} standard deviation (false results and outliers excluded)
- u_{PT} standard uncertainty of the assigned value (false results and outliers excluded)
- F number of false positive or false negative results
- < number of low outliers
- > number of high outliers
-  results deviating more than 1 s from m, or unusually many deviating results.

Figure legends

-  results within the interval of acceptance
-  outlier
-  false negative result
- * value outside the x-axis scale

Results

General outcome

Samples were sent to 167 participants; 39 in Sweden, 113 in European countries, and 15 outside of Europe. Of the 153 participants that reported results, 95 (62 %) provided at least one result that received an annotation. In the previous PT round with similar analyses (April 2021) the proportion was 69 %.

Individual results are listed in Annex 1 and on the website: <https://www2.slv.se/absint>. Z-scores for individual results are listed in Annex 2.

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												
	0 annotations	1 annotation	2 annotations	>2 annotations	0 annotations	1 annotation	2 annotations	>2 annotations	0 annotations	1 annotation	2 annotations	>2 annotations
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 hydrophila</i> <i>Clostridium bifermentans</i> <i>Escherichia coli</i> <i>Hafnia alvei</i> <i>Lactobacillus plantarum</i> <i>Staphylococcus aureus</i>				<i>Clostridium perfringens</i> <i>Hanseniopsis uvarum</i> <i>Serratia marcescens</i> <i>Shewanella putrefaciens</i> <i>Staphylococcus aureus</i>			
Analysis	Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Aerobic micro-organisms, 30 °C	All	147	0%	1%	All	148	0%	3%	<i>S. putrefaciens</i> <i>S. aureus</i>	148	0%	3%
Psychrotrophic micro-organisms	All	15	-	-	All	15	7%	0%	<i>S. putrefaciens</i> <i>S. aureus</i>	15	13%	0%
Enterobacteriaceae	<i>E. coli</i>	126	0%	1%	<i>E. coli</i> <i>H. alvei</i>	127	1%	2%	<i>S. marcescens</i>	127	11%	3%
<i>E. coli</i>	<i>E. coli</i>	103	7%	3%	<i>E. coli</i>	103	0%	5%	-	104	2%	0%
Presumptive <i>B. cereus</i>	<i>B. cereus</i>	108	4%	8%	(<i>S. aureus</i>) (<i>A. hydrophila</i>)	108	6%	0%	(<i>S. aureus</i>) (<i>S. marcescens</i>)	108	3%	0%
Coagulase-positive staphylococci	(<i>S. xylosum</i>)	95	2%	0%	<i>S. aureus</i>	94	1%	7%	<i>S. aureus</i>	94	15%	1%
Lactic acid bacteria	<i>L. plantarum</i>	49	2%	2%	<i>L. plantarum</i>	49	0%	4%	(<i>S. aureus</i>)	48	23%	0%
<i>C. perfringens</i>	-	47	0%	0%	(<i>C. bifermentans</i>)	47	28%	0%	<i>C. perfringens</i>	47	6%	4%
Anaerob. sulphite red. bacteria	-	57	0%	0%	<i>C. bifermentans</i>	55	2%	2%	<i>C. perfringens</i>	55	4%	0%
Aerobic microorg. in fish products	All	20	0%	0%	All	20	0%	0%	<i>S. putrefaciens</i> <i>S. aureus</i>	20	0%	10%
H ₂ S-prod. bacteria in fish products	-	19	5%	0%	<i>H. alvei</i>	19	0%	0%	<i>S. putrefaciens</i>	19	-	-
Yeasts	<i>K. marxianus</i>	123	2%	5%	-	123	2%	0%	<i>H. uvarum</i>	124	0%	5%
Moulds	<i>C. cladosporioides</i>	121	7%	5%	-	121	2%	0%	-	120	6%	0%

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

■ The results are not evaluated

Aerobic microorganisms 30 °C

Sample A

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

One low outlier was reported.

Sample B

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

Four low outliers were reported.

Sample C

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

Two low and three high outliers were reported.

General remarks

Most participants followed either NMKL 86:2013, ISO 4833-1:2013 or used 3M Petrifilm AC. The withdrawn NMKL 86:2006 and ISO 4833:2003 were used by eleven and five participants, respectively.

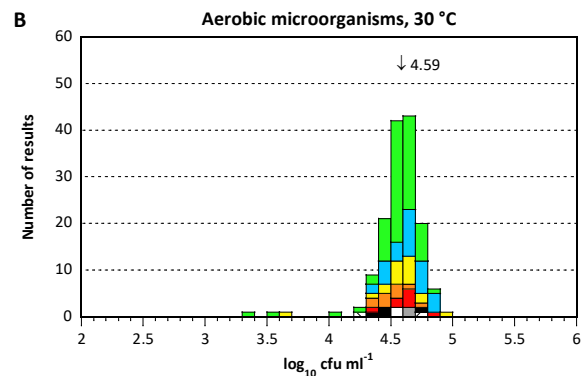
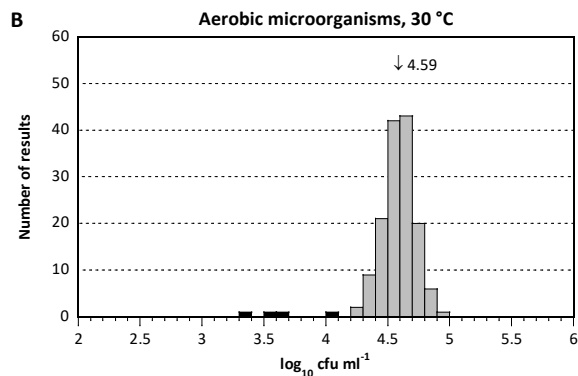
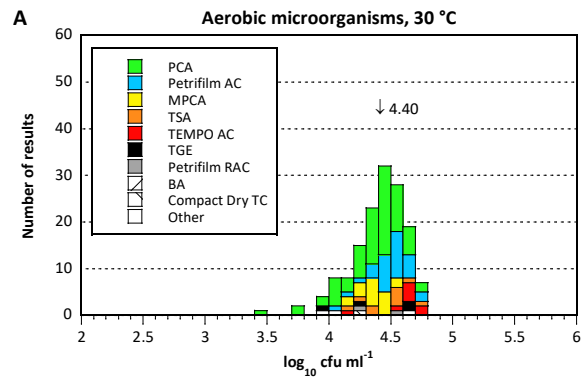
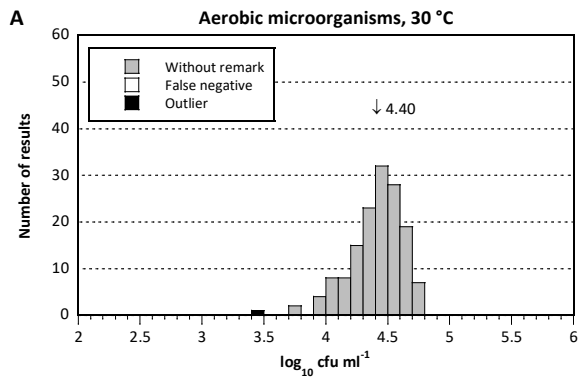
Both NMKL 86:2013 and ISO 4833-1:2013 are based on incubation on PCA or MPCA at 30 °C for 72 h. Users of Petrifilm AC can use different times/temperatures, depending on the method validation. For example, AOAC[®] prescribes incubation at 35 °C for 48 h while AFNOR prescribes 30 °C for either 48 h or 72 h, depending on which product that is analysed. ISO 4833-1:2013 was last reviewed by ISO in 2019 and remains current. An amendment with a clarification on the scope of the method is available (ISO 4833-1:2013/Amd 1:2022).

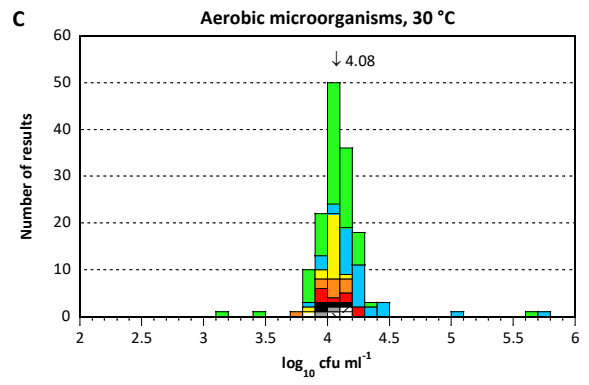
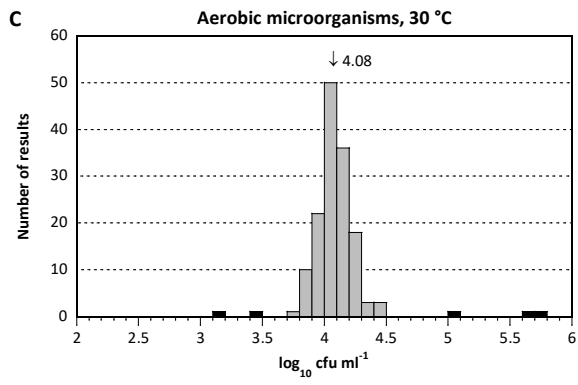
The majority of the participants incubated on PCA, but Petrifilm AC was also common. Incubation on MPCA was mainly done by laboratories within the dairy industry. Incubation on TSA was mainly done by users of a company-specific method. A few participants used TEMPO AC, which is based on MPN (Most Probable 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.

Comment: One participant followed ISO 13559/IDF 153 (contaminating microorganisms). Since the participant incubated on PCA, the results were still included in the evaluation.

Table 2. 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	146	4.40	0.21	0	1	0	148	144	4.59	0.13	0	4	0	148	143	4.08	0.13	0	2	3
PCA	70	69	4.36	0.22	0	1	0	70	67	4.58	0.11	0	3	0	70	67	4.06	0.11	0	2	1
Petrifilm AC	31	31	4.49	0.15	0	0	0	32	32	4.63	0.14	0	0	0	32	30	4.18	0.15	0	0	2
MPCA	18	18	4.35	0.12	0	0	0	18	17	4.60	0.13	0	1	0	18	18	4.03	0.06	0	0	0
TSA	10	10	4.47	0.20	0	0	0	10	10	4.51	0.13	0	0	0	10	10	4.03	0.12	0	0	0
TEMPO AC	8	8	4.59	0.20	0	0	0	8	8	4.61	0.15	0	0	0	8	8	4.08	0.11	0	0	0
TGE	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Petrifilm RAC	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
Compact Dry TC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0





Psychrotrophic microorganisms

Sample A

The strains of *B. cereus*, *L. plantarum*, *E. coli* and *S. xylosus* were present in the highest concentrations and were the main target organisms. In the quality control at the Swedish Food Agency (ten days incubation on PCA at 6.5 °C), no colonies were detected. In previous tests of the same sample, a larger than expected variation was found among the results, which meant that the requirements for homogeneity were not met.

In total, 13 positive results and two zero results were reported. The two zero results were reported by participants that incubated at 6.5–7 °C.

Due to the varying results in the quality control, the results for sample A are not evaluated, and no z-scores are calculated for the analysis.

Sample B

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

One false negative result was reported.

Sample C

The strains of *S. aureus* and *S. putrefaciens* were target organisms.

Two false negative results were reported.

General remarks

In total, 15 participants reported results. Eleven of these (73 %) incubated on PCA. Petrifilm AC, MPCA and Long & Hammer agar were used by two, one and one participants, respectively.

The methods used by the participants differ considerably in the incubation conditions. Users of NMKL 86:2013 typically incubated for 10 days at 6.5 °C, but 20 h at 17 °C followed by 3 days at 7 °C was also 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 participants 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, medium or incubation. The results are therefore difficult to evaluate. 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.

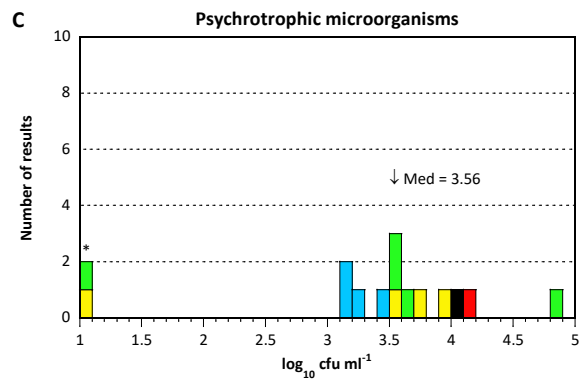
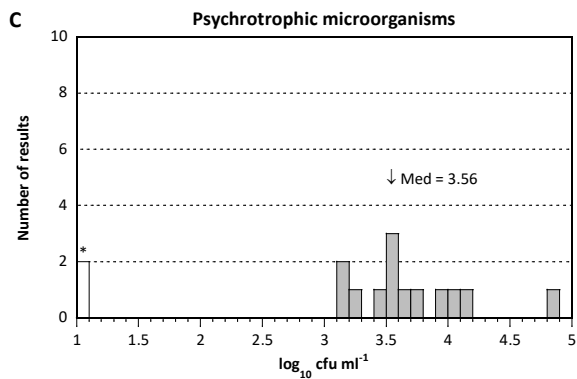
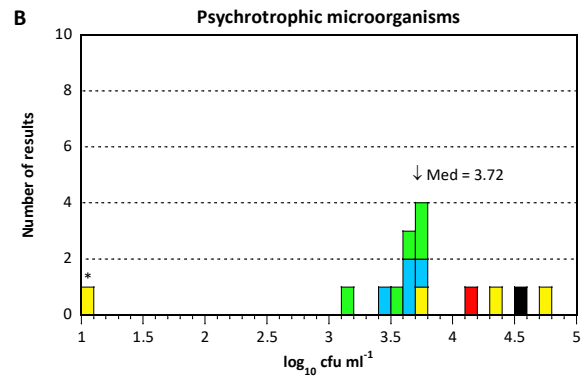
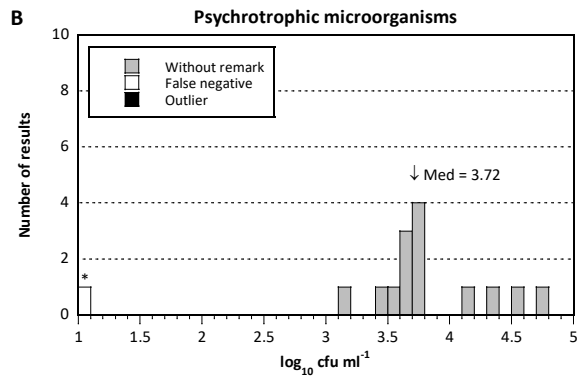
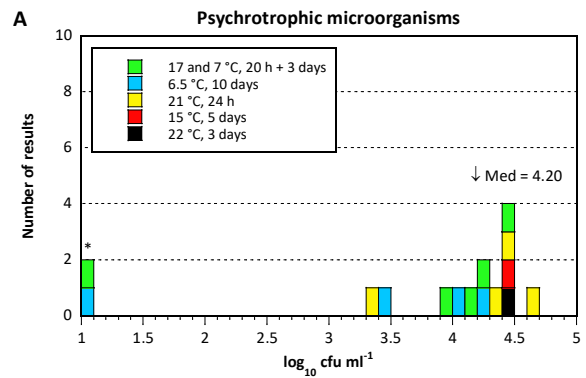
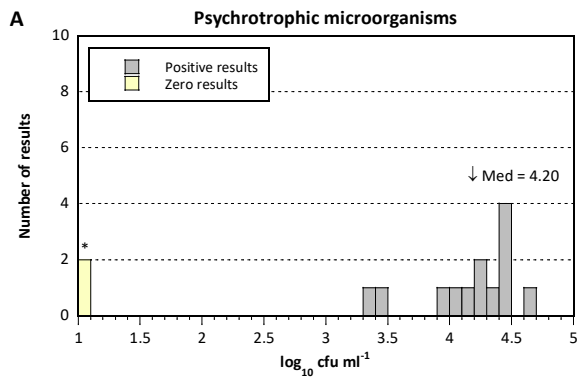
Table 3. Results from analysis of psychrotrophic microorganisms

Incubation	Sample A ¹							Sample B							Sample C						
	N	n	Med ²	s	F	<	>	N	n	Med ²	s	F	<	>	N	n	Med ²	s	F	<	>
All results	15	0	4.20	1.51	-	-	-	15	14	3.72	0.44	1	0	0	15	13	3.56	0.48	2	0	0
17 and 7 °C, 20 h + 3 days ³	5	0	-	-	-	-	-	5	5	3.61	0.23	0	0	0	5	4	-	-	1	0	0
6.5 °C, 10 days	4	0	-	-	-	-	-	4	4	-	-	0	0	0	4	4	-	-	0	0	0
21 °C, 24 h	4	0	-	-	-	-	-	4	3	-	-	1	0	0	4	3	-	-	1	0	0
15 °C, 5 days	1	0	-	-	-	-	-	1	1	-	-	0	0	0	1	1	-	-	0	0	0
22 °C, 3 days	1	0	-	-	-	-	-	1	1	-	-	0	0	0	1	1	-	-	0	0	0

¹ The results for sample A are not evaluated

² Median

³ Includes one participant that used 20 °C for 20 h followed by 7 °C for 3 days.



Enterobacteriaceae

Sample A

The strain of *E. coli* was target organism. On VRBG, it forms typical red/purple colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative.

One low outlier was reported.

Sample B

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 one false negative result.

Sample C

The strain of *S. marcescens* was target organism. On VRBG, it may form smaller colonies that are surrounded by a less prominent bile salt precipitation zone.

Two low and two high outliers were reported, as well as 14 false negative results.

The false negative results were mainly correlated with the use of Petrifilm EB. Of the 34 participants that used Petrifilm EB, 11 (32 %) reported a 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 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.

Most participants followed either NMKL 144:2005 (40 %) or a method with Petrifilm EB (27 %), while the ISO methods (various versions) were used by 21 %. 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.

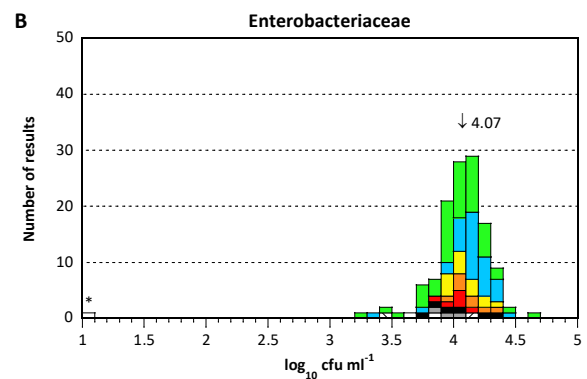
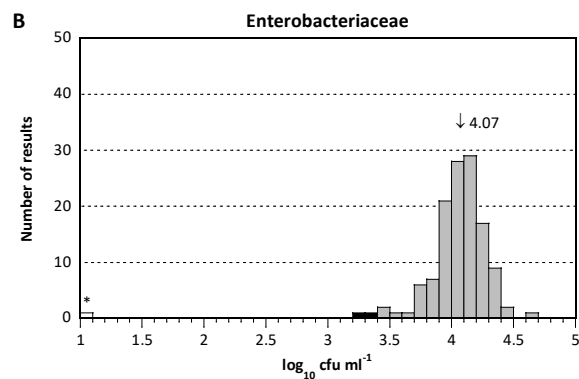
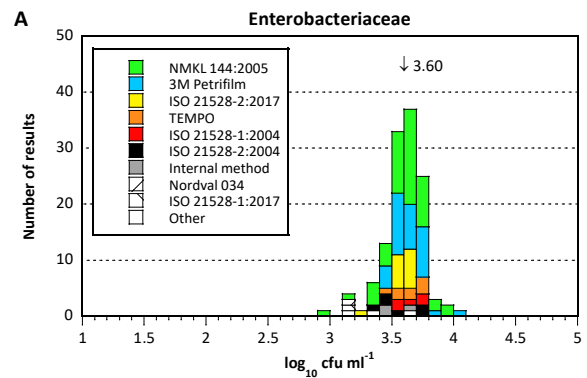
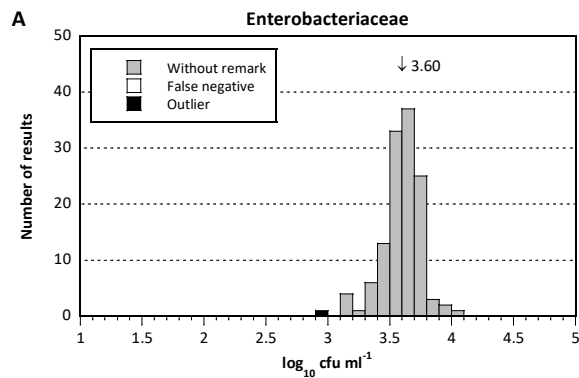
The number of users of ISO 21528-2:2017 was higher compared to the withdrawn ISO 21528-2:2004 (14 and 6 participants, respectively). In contrast, six participants followed the withdrawn ISO 21528-1:2004, while only one had adopted the new ISO 21528-1:2017. The reported results from the different ISO methods were similar.

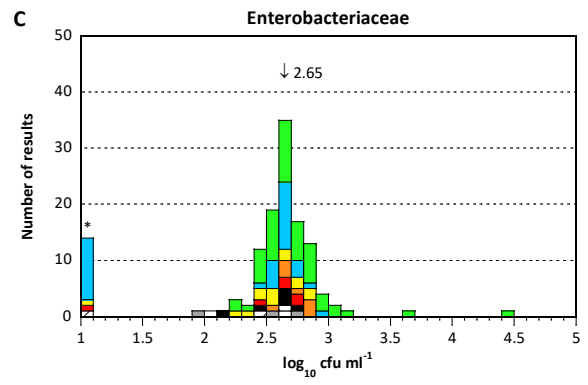
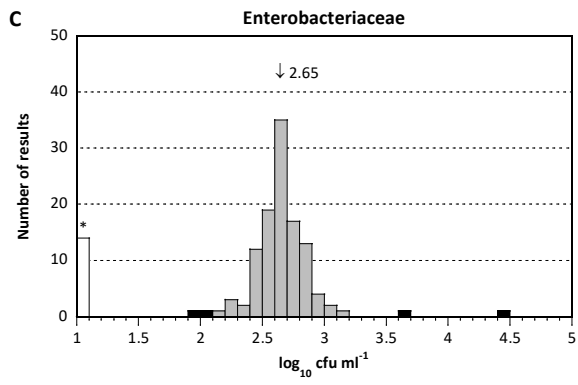
NMKL 144:2005 stipulates confirmation of presumptive colonies with an oxidase test. ISO 21528-2:2017 stipulates confirmation of presumptive colonies with both an oxidase test and with a test

for glucose fermentation. Here, the majority of the participants that performed a confirmation test specified that it consisted of an oxidase test.

Table 4. Results from analysis of Enterobacteriaceae.

Method	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	126	125	3.60	0.15	0	1	0	127	124	4.07	0.19	1	2	0	127	109	2.65	0.17	14	2	2
NMKL 144:2005	51	50	3.61	0.16	0	1	0	51	50	4.04	0.20	0	1	0	51	49	2.67	0.20	0	0	2
3M Petrifilm	34	34	3.63	0.11	0	0	0	34	33	4.16	0.13	0	1	0	34	23	2.66	0.10	11	0	0
ISO 21528-2:2017	14	14	3.59	0.11	0	0	0	14	14	4.08	0.14	0	0	0	14	13	2.59	0.18	1	0	0
TEMPO	8	8	3.64	0.11	0	0	0	8	8	4.13	0.11	0	0	0	8	8	2.71	0.10	0	0	0
ISO 21528-1:2004	5	5	3.64	0.11	0	0	0	6	6	4.02	0.11	0	0	0	6	5	2.64	0.12	1	0	0
ISO 21528-2:2004	6	6	3.55	0.13	0	0	0	6	6	4.02	0.22	0	0	0	6	6	2.57	0.23	0	0	0
Internal method	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	2	-	-	0	1	0
Nordval 034	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	1	-	-	1	0	0
ISO 21528-1:2017	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	1	-	-	1	0	0	2	1	-	-	0	1	0





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/VRB. The strain of *E. coli* produces both gas and indole in LTLSB. It is also positive for β -glucuronidase.

One low and two high outliers were reported, as well as seven false negative results. The false negative results do not appear to be correlated with any specific method or medium.

Sample B

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 LTLSB.

Four low and one high outliers were reported.

Sample C

No target organism was present in the sample.

Two false positive results were reported.

General remarks

In total, 35 % of the participants used a method based on 3M™ Petrifilm™. NMKL 125:2005 and ISO 16649-2:2001 were in comparison used by 26 % and 16 % of the participants, respectively. A few of the participants that followed NMKL 125:2005 and ISO 16649-2:2001 used media other than those stipulated by the respective standards, for example Petrifilm EC/CC or Brilliance EC/CC. 16649-2:2001 was last reviewed by ISO in 2019, and remains current. NMKL 125 is scheduled for revision, and the new version will likely be more similar to ISO 16649-2.

ISO 7251:2005 and NMKL 96:2009 were used by two and one participants, respectively. 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. ISO 7251:2005 was last reviewed by ISO in 2019, and remains current.

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*.

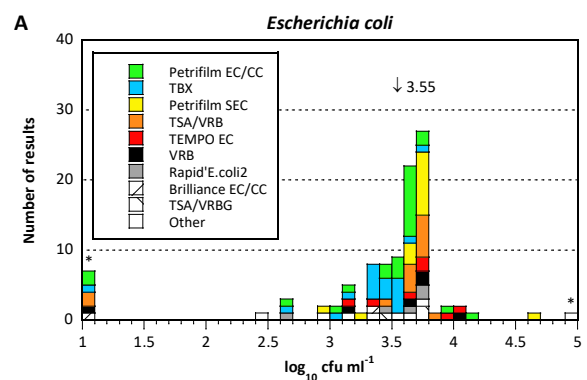
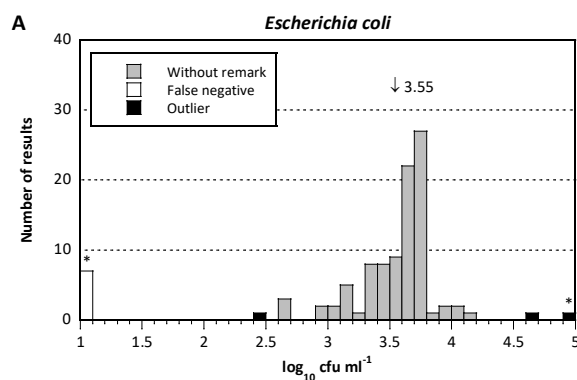
In general, confirmation appears to have been performed when required by the method. For example, 81 % of the participants that followed NMKL 125:2005 performed a confirmation test.

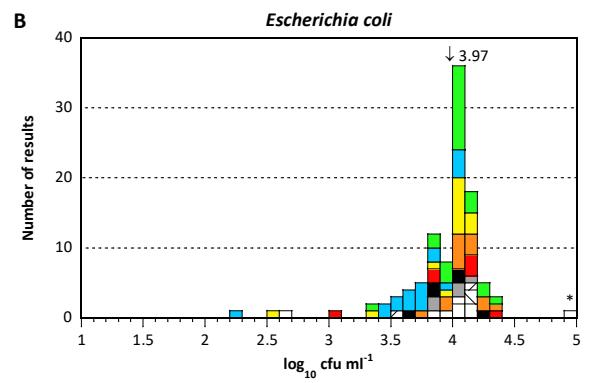
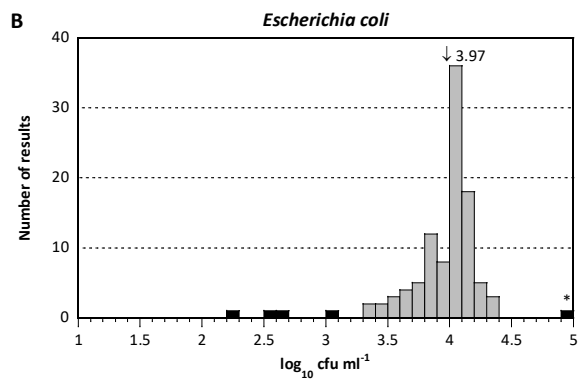
Altogether, the results from the different methods and media were similar. The only notable difference was that the results for TBX were somewhat lower compared to other media, though the difference was within one standard deviation. Similar differences have been observed in several previous PT rounds, and can therefore be considered normal.

Due to method differences, incubation was either at 41.5–44 °C (51 %) or at 35–37 °C (49 %). The mean values, and the number of false results and outliers did not differ notably for the two temperature groups, for any of the samples.

Table 5. 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	103	93	3.55	0.28	7	1	2	103	98	3.97	0.21	0	4	1	104	102	-	-	2	-	-
Petrifilm EC/CC	24	22	3.56	0.29	2	0	0	24	24	4.02	0.19	0	0	0	24	22	-	-	2	-	-
TBX	19	18	3.39	0.25	1	0	0	19	18	3.76	0.20	0	1	0	19	19	-	-	0	-	-
Petrifilm SEC	15	14	3.63	0.25	0	0	1	15	14	3.98	0.21	0	1	0	15	15	-	-	0	-	-
TSA/VRB	14	12	3.68	0.09	2	0	0	14	14	4.08	0.16	0	0	0	14	14	-	-	0	-	-
TEMPO EC	7	7	3.64	0.33	0	0	0	7	6	4.07	0.21	0	1	0	7	7	-	-	0	-	-
VRB	6	5	3.67	0.35	1	0	0	6	6	3.95	0.21	0	0	0	6	6	-	-	0	-	-
Rapid'E.coli 2	5	5	3.45	0.43	0	0	0	5	5	3.98	0.15	0	0	0	5	5	-	-	0	-	-
Brilliance EC/CC	3	2	-	-	1	0	0	3	3	-	-	0	0	0	3	3	-	-	0	-	-
TSA/VRBG	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	-	-
Other	7	5	-	-	0	1	1	7	5	-	-	0	1	1	8	8	-	-	0	-	-





Presumptive *Bacillus cereus*

Sample A

The strain of *B. cereus* was target organism, but the strains of *E. coli*, *L. plantarum* and *S. xylosus* 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.

Nine low outliers were reported, as well as four false negative results. Participants that used Compact Dry X-BC appear to be over-represented among the deviating results. In at least one instance this was (according to the participant) correlated with the appearance of atypical white colonies on the Compact Dry X-BC plates. Still, previous correspondence with other participants (that have reported accepted results for the particular strain of *B. cereus*) indicate that it is capable of forming typical blue colonies on Compact Dry X-BC.

Sample B

No target organism was present in the sample. Several strains in the sample may however form 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 participants reported a false positive result.

Sample C

No target organism was present in the sample. *S. aureus* and *S. marcescens* may sometimes form atypical colonies on BcsA. At the Swedish Food Agency, colonies were observed on BA – when transferred to BcsA they however displayed an atypical morphology and did not have a blue colour.

Three participants reported a false positive result.

General remarks

Most participants followed either NMKL 67:2010 (42 %) or ISO 7932:2004 (30 %). The new NMKL 67:2021 – which replaces NMKL 67:2010 – was in contrast only followed by five participants (5 %). With NMKL 67:2021, primary incubation on BcsA is followed by confirmation on BA. With ISO 7932:2004, primary incubation on MYP is followed by confirmation on BA. An amendment is available for the ISO method (ISO 7932:2004/Amd 1:2020). It contains optional tests, including for PCR detection of *cytK* genes.

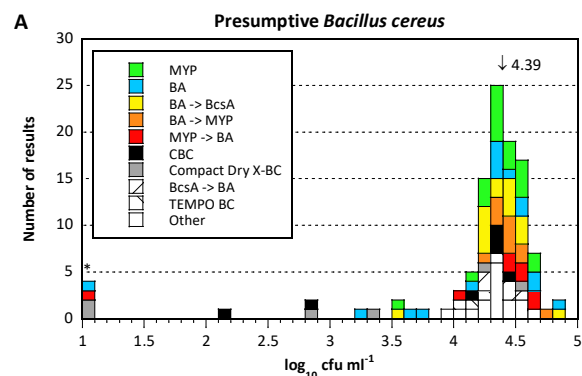
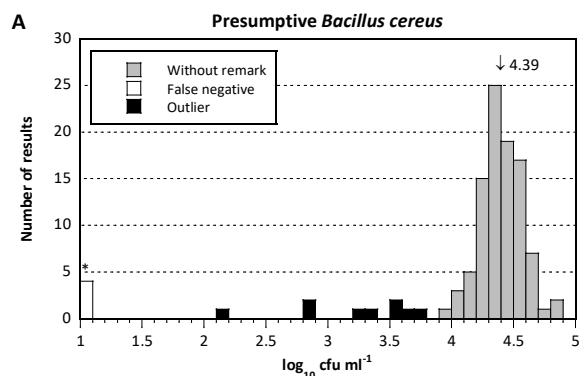
On BA, *B. cereus* forms large, irregular grey colonies, surrounded by a distinct zone of haemolysis. On BcsA, presumptive *B. cereus* form bluish colonies that are surrounded by a blue zone of precipitation, due to lecithinase activity on egg yolk present in the medium. On MYP, presumptive *B. cereus* form large pink colonies that are normally surrounded by a zone of precipitation, again as a consequence of lecithinase activity.

Compact Dry X-BC was used by six participants. The chromogenic and selective agents in this medium cause *B. cereus* to form blue/green colonies, whereas other bacteria normally form white colonies. For sample A, two false negative results and two low outliers were reported by users of this method. Somewhat lower results are not unexpected for Compact Dry X-BC compared to the reference method ISO 7932:2004, and is mentioned in both the NordVal 045 and MicroVal 2011-LR41 validations. It has also been seen in previous PT rounds by the Swedish Food Agency, for example when the same strain of *B. cereus* was included in PT Food April 2021 and PT Food October 2021. Slightly lower results for Compact Dry X-BC can therefore be considered normal. False negative results should however be of concern for the affected laboratories.

The chromogenic medium CBC was used by seven participants. CBC contains the substrate X-Gluc, which is cleaved by *B. cereus* β -glucuronidase. Colonies of *B. cereus* are therefore white with a blue/green centre on this medium. Similar to Compact Dry X-BC, relatively many low outliers were reported for CBC in sample A. Whether this is a random occurrence or possibly due to differences in the method/medium itself is difficult to determine.

Table 6. 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	108	95	4.39	0.17	4	9	0	108	101	-	-	7	-	-	108	105	-	-	3	-	-
MYP	20	19	4.40	0.15	0	1	0	19	19	-	-	0	-	-	19	18	-	-	1	-	-
BA	15	11	4.46	0.17	1	3	0	16	13	-	-	3	-	-	16	15	-	-	1	-	-
BA → BcsA	16	15	4.41	0.16	0	1	0	16	16	-	-	0	-	-	16	15	-	-	1	-	-
BA → MYP	11	11	4.45	0.14	0	0	0	11	11	-	-	0	-	-	11	11	-	-	0	-	-
MYP → BA	8	7	4.48	0.19	1	0	0	8	8	-	-	0	-	-	8	8	-	-	0	-	-
CBC	7	5	4.33	0.14	0	2	0	7	7	-	-	0	-	-	7	7	-	-	0	-	-
Compact Dry X-BC	6	2	-	-	2	2	0	6	5	-	-	1	-	-	6	6	-	-	0	-	-
BcsA → BA	6	6	4.42	0.15	0	0	0	6	6	-	-	0	-	-	6	6	-	-	0	-	-
TEMPO BC	5	5	-	-	0	0	0	5	5	-	-	0	-	-	5	5	-	-	0	-	-
Other	14	14	-	-	0	0	0	14	11	-	-	3	-	-	14	14	-	-	0	-	-



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.

Two false positive results were reported.

Sample B

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

Five low and two high outliers were reported, as well as one false negative result. Three of the five low outliers were reported by participants that used TEMPO STA. The other two results reported by users of TEMPO STA were also low, though within the acceptance limits.

Sample C

The strain of *S. aureus* was target organism. At the Swedish Food Agency, the growth of *S. aureus* on RPFA was in one test slow and notably not all colonies had a typical appearance and/or a distinct precipitation zone after 48 h incubation at 37 °C. Extended incubation for 72 h allowed typical colonies to form on the plates. In follow-up analyses, typical colonies appeared on RPFA after 48 h.

One low outlier was reported, as well as 14 false negative results. Thirteen of the false negative results were from participants that used BP without RPF. All of the 13 participants performed some kind of confirmation. The false negative results could not be attributed to the use of a particular confirmation test. Tube coagulase test, slide coagulase test, VITEK, RPFA, and Oxoid Dryspot Staphytest Plus were all represented as confirmation methods used by the participants that reported false negative results.

General remarks

Most participants (45 %) followed NMKL 66:2009. Other major methods were 3M™ Petrifilm™ (15 %), ISO 6888-1:1999 (13 %) and ISO 6888-2:1999 (6 %). Both of the ISO methods are now withdrawn and replaced by ISO 6888-1:2021 and ISO 6888-2:2021, respectively. One participant 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 RPFA. In comparison, ISO 6888-1:2021 stipulates surface spreading on BP, whereas 6888-2:2021 stipulates the use of RPFA. 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 RPFA, 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.

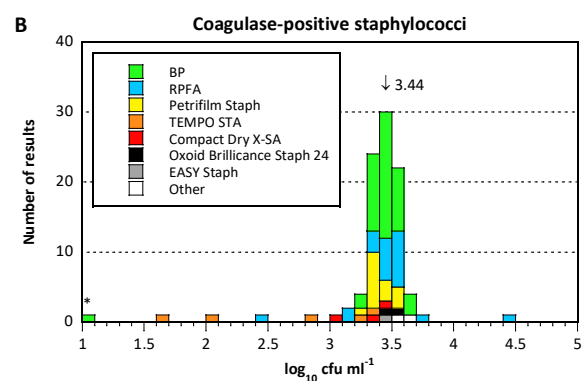
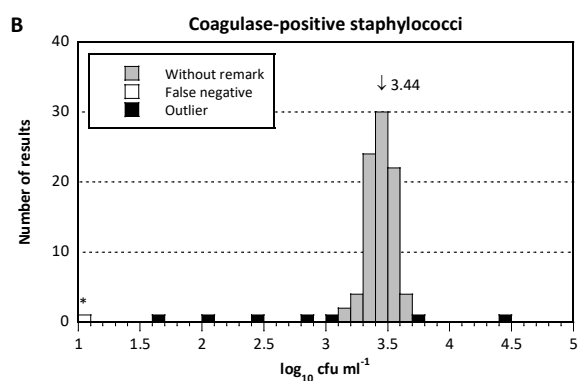
Taken together, the results were for the most part similar for BP, RPFA and Petrifilm Staph, in all three samples. The mean value was slightly lower for Petrifilm Staph in sample B, but this has been seen also in previous PT rounds and can be considered normal.

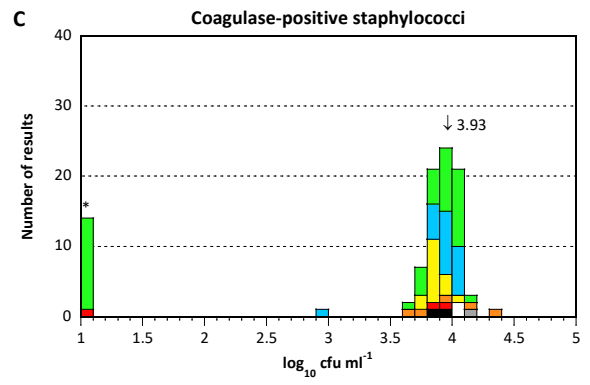
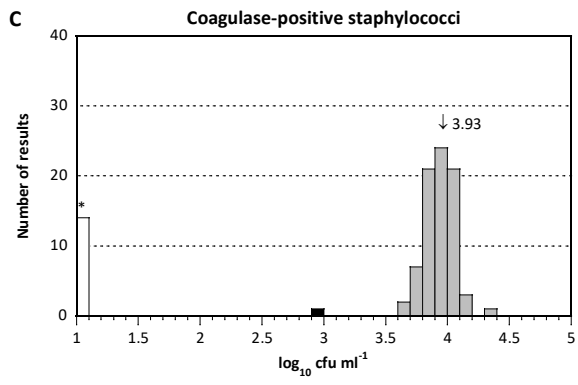
TEMPO STA, Compact Dry™ X-SA, Brilliance™ Staph 24 and EASY Staph® were only used by a small number of participants, which makes them difficult to evaluate. One participant used BA, in combination with StafChrom.

In total, 73 % of the participants stated that they performed some kind of confirmation test. Overall, most participants performed a tube coagulase test, though users of Petrifilm Staph mainly used Petrifilm Disk for the 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.

Table 7. 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	95	93	-	-	2	-	-	94	86	3.44	0.10	1	5	2	94	79	3.93	0.12	14	1	0
BP	45	44	-	-	1	-	-	44	43	3.45	0.09	1	0	0	44	31	3.93	0.11	13	0	0
RPFA	22	22	-	-	0	-	-	22	19	3.44	0.13	0	1	2	22	21	3.95	0.08	0	1	0
Petrifilm Staph	15	14	-	-	1	-	-	15	15	3.40	0.08	0	0	0	15	15	3.87	0.08	0	0	0
TEMPO STA	5	5	-	-	0	-	-	5	2	-	-	0	3	0	5	5	3.96	0.29	0	0	0
Compact Dry X-SA	3	3	-	-	0	-	-	3	2	-	-	0	1	0	3	2	-	-	1	0	0
Oxoid Brilliance Staph 24	2	2	-	-	0	-	-	2	2	-	-	0	0	0	2	2	-	-	0	0	0
EASY Staph®	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	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 outlier was reported, as well as one false negative result.

Sample B

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.

Two low outliers were reported.

Sample C

No target organism was present in the sample.

Eleven false positive result were reported. These are likely due to detection of *S. aureus*. Strains of *S. aureus* have in previous PT rounds formed small colonies on MRS and MRS-aB. In the initial quality control at the Swedish Food Agency, small transparent colonies were observed on MRS-aB at a concentration of $3.85 \log_{10} \text{ cfu ml}^{-1}$. In the subsequent confirmation these were catalase positive, and they were therefore not considered as lactic acid bacteria.

Five of the 11 false positive results (45 %) were from participants that performed a confirmation.

General remarks

Most of the participants followed NMKL 140, either NMKL 140:2007 (27 %), or the older NMKL 140:1991 (6 %). The older method prescribes spreading onto MRS-S, whereas the new method prescribes MRS-aB. In comparison, ISO 15214:1998, which was used by 16 % of the participants, 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 continues to increase, and the method was here used by 21 % of the participants. Two participants stated ISO 7889/IDF 117:2003, 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.

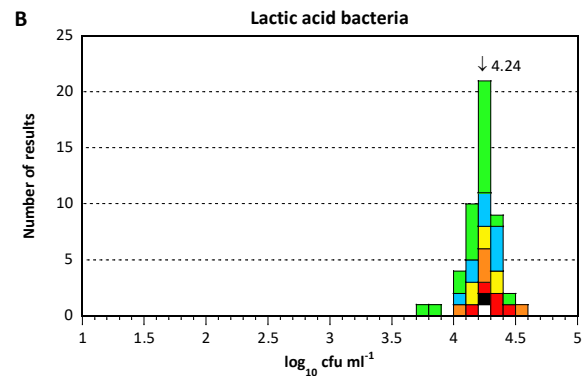
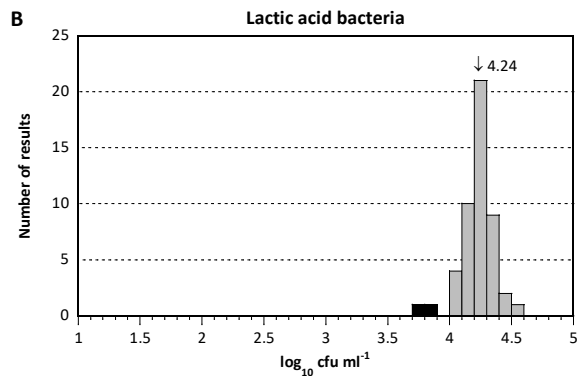
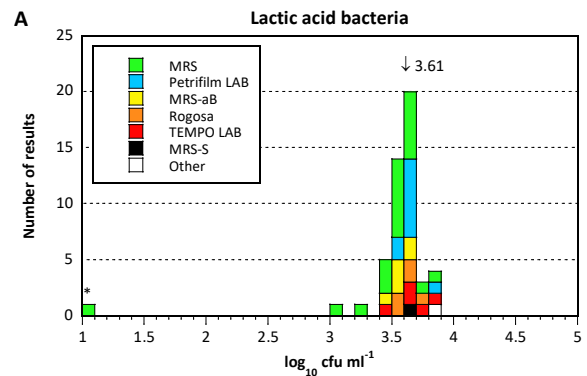
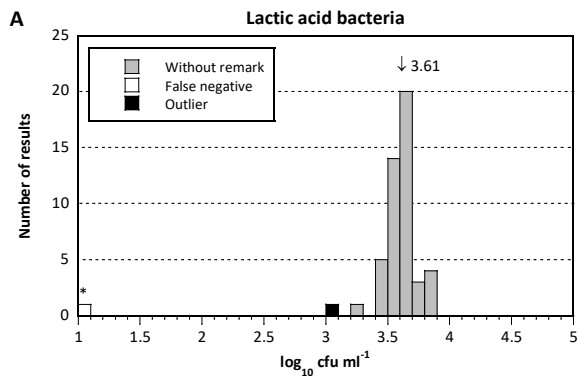
Lactic acid bacteria constitute a heterogeneous group of microorganisms, and therefore have different optimal medium, pH and incubation conditions. For example, MRS-aB (pH 6.2) is a less selective medium that allows the growth of a more wide range of lactic acid bacteria. This may however also

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. Lactic acid bacteria are Gram positive and normally catalase-negative. Confirmation was here performed by roughly half (49 %) of the participants. Usually, it consisted of a catalase test or microscopy. Overall, the use of a confirmation test does not appear to have had an impact on the result.

Table 8. 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	49	47	3.61	0.12	1	1	0	49	47	4.24	0.11	0	2	0	48	37	-	-	11	-	-
MRS	21	19	3.58	0.13	1	1	0	21	19	4.22	0.09	0	2	0	20	15	-	-	5	-	-
Petrifilm LAB	10	10	3.64	0.07	0	0	0	10	10	4.24	0.10	0	0	0	10	7	-	-	3	-	-
MRS-aB	6	6	3.56	0.06	0	0	0	6	6	4.26	0.10	0	0	0	6	4	-	-	2	-	-
Rogosa	5	5	3.62	0.09	0	0	0	5	5	4.27	0.18	0	0	0	5	5	-	-	0	-	-
TEMPO LAB	5	5	3.67	0.15	0	0	0	5	5	4.32	0.12	0	0	0	5	5	-	-	0	-	-
MRS-S	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	-	-
Other	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	1	-	-



Clostridium perfringens

Sample A

No target organism was present in the sample.

All 47 results were correct negative.

Sample B

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.

Thirteen participants reported a false positive result. The reported concentrations indicate that the false positive results are due to detection of *C. bifermentans*.

Sample C

The strain of *C. perfringens* was target organism.

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

General remarks

Most participants followed either NMKL 95:2009 (64 %) or ISO 7937:2004 (26 %). One participant followed the older NMKL 95:1997 and two participants stated that they analysed according to NMKL 56:2015 (Sulphite-reducing Clostridia). 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.

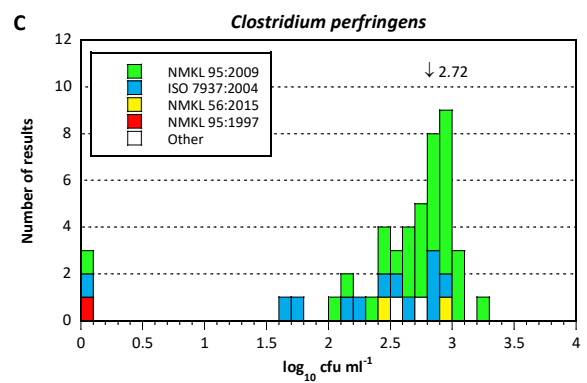
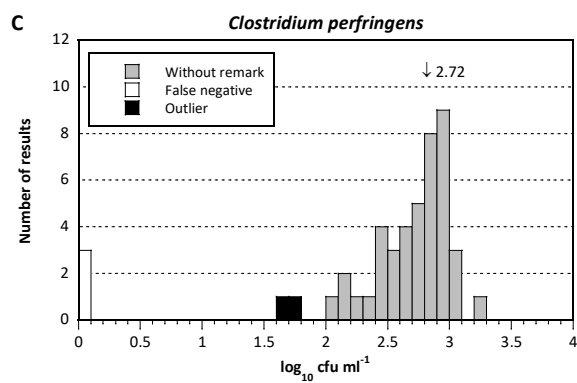
In line with ISO 7937:2004 and NMKL 95:2009, the majority (89 %) of the participants reported the use of TSC. On TSC, *C. perfringens* form black colonies after anaerobic incubation at 37 °C. The media ISA, SC and mCP were used by two, one and one participant, respectively. Comparisons with TSC are difficult to make – due to the low number of users of these media – but TSC has by some been recommended 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, 88 % of the participants 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 participants (91 %) incubated at 37 °C, while only a few (9 %) incubated at 44 °C. It is therefore difficult to say if the choice of incubation temperature had an impact on the outcome, but it does not appear to be the case.

Table 9. Results from analysis of *Clostridium perfringens*.

Method	Sample A						Sample B						Sample C								
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	47	47	-	-	0	-	-	47	34	-	-	13	-	-	47	42	2.72	0.27	3	2	0
NMKL 95:2009	30	30	-	-	0	-	-	30	23	-	-	7	-	-	30	29	2.76	0.27	1	0	0
ISO 7937:2004	12	12	-	-	0	-	-	12	10	-	-	2	-	-	12	9	2.61	0.28	1	2	0
NMKL 56:2015	2	2	-	-	0	-	-	2	1	-	-	1	-	-	2	2	-	-	0	0	0
NMKL 95:1997	1	1	-	-	0	-	-	1	0	-	-	1	-	-	1	0	-	-	1	0	0
Other	2	2	-	-	0	-	-	2	0	-	-	2	-	-	2	2	-	-	0	0	0



Anaerobic sulphite-reducing bacteria

Sample A

No target organism was present in the sample.

All results were correct negative.

Sample B

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

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

Sample C

The strain of *C. perfringens* was target organism.

Two false negative results were reported.

General remarks

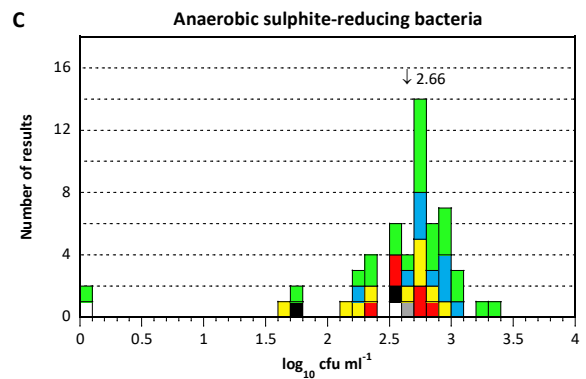
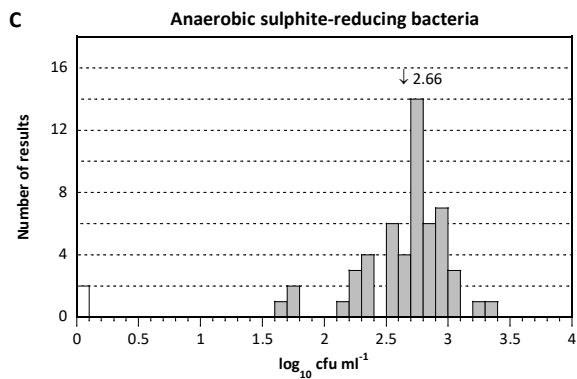
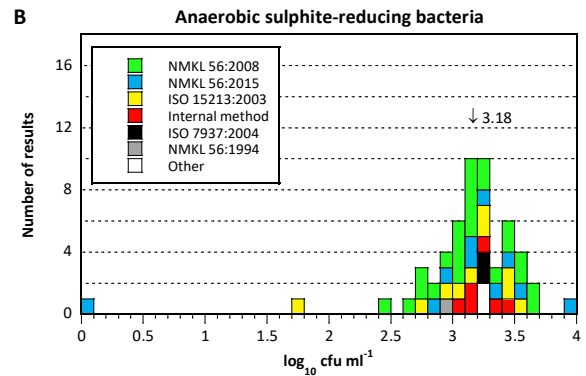
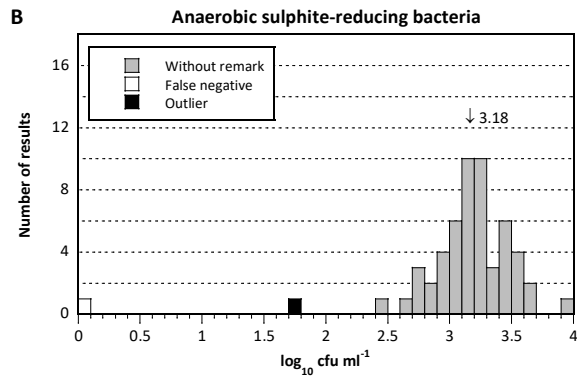
The majority of the participants (63 %) followed a version of NMKL 56. However only 18 % followed the current NMKL 56:2015. Instead, most participants still followed either NMKL 56:2008 (44 %) or the considerably older NMKL 56:1994 (4 %). ISO 15213:2003 was used by 18 % of the participants. This was last reviewed by ISO in 2015, and remains current. It is however scheduled to be replaced by ISO 15213-1 ("Enumeration of sulphite-reducing *Clostridium* spp. by colony-count technique"), which is currently under development. Two participants 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 consequently the medium most frequently used by the participants (42 %). 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, participants reported using TSC (25 %), SFP (16 %), TS (5 %) and PAB (4 %). 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 however not cause any obvious problems here.

Table 10. Results from analysis anaerobic sulphite-reducing bacteria.

Method	Sample A						Sample B						Sample C						
	N	n	m	s	F	< >	N	n	m	s	F	< >	N	n	m	s	F	< >	
All results	57	57	-	-	0	- -	55	53	3.18	0.28	1	1 0	0	55	53	2.66	0.34	2	0 0
NMKL 56:2008	24	24	-	-	0	- -	24	24	3.13	0.31	0	0 0	0	24	23	2.73	0.34	1	0 0
NMKL 56:2015	10	10	-	-	0	- -	10	9	3.28	0.33	1	0 0	0	10	10	2.79	0.22	0	0 0
ISO 15213:2003	10	10	-	-	0	- -	10	9	3.20	0.26	0	1 0	0	10	10	2.49	0.39	0	0 0
Internal method	6	6	-	-	0	- -	6	6	3.24	0.15	0	0 0	0	6	6	2.63	0.20	0	0 0
ISO 7937:2004	2	2	-	-	0	- -	2	2	-	-	0	0 0	0	2	2	-	-	0	0 0
NMKL 56:1994	2	2	-	-	0	- -	1	1	-	-	0	0 0	0	1	1	-	-	0	0 0
Other	3	3	-	-	0	- -	2	2	-	-	0	0 0	0	2	1	-	-	1	0 0



Aerobic microorganisms in fish products, 20–25 °C

Sample A

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

No outliers or false negative results were reported.

Sample C

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

One low and one high outliers were reported.

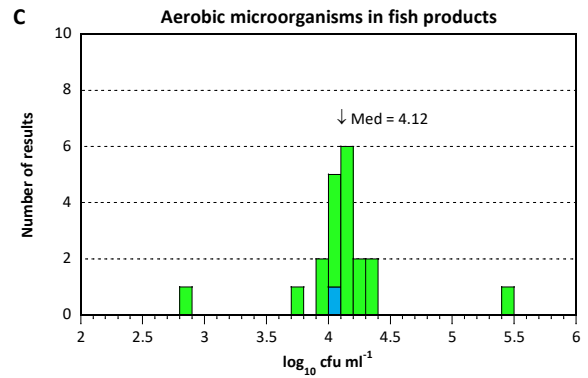
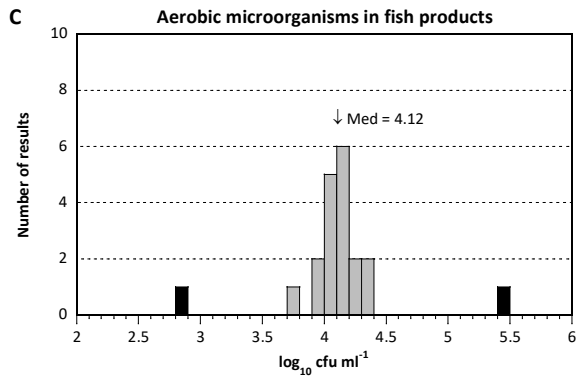
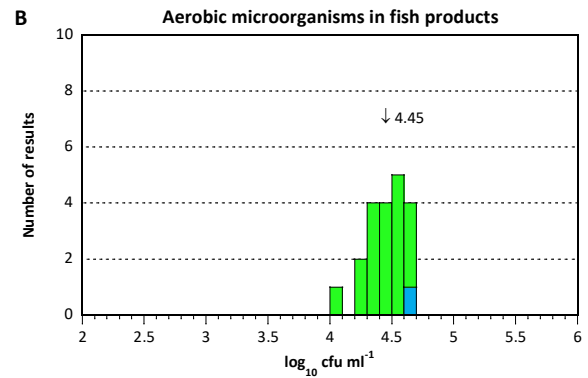
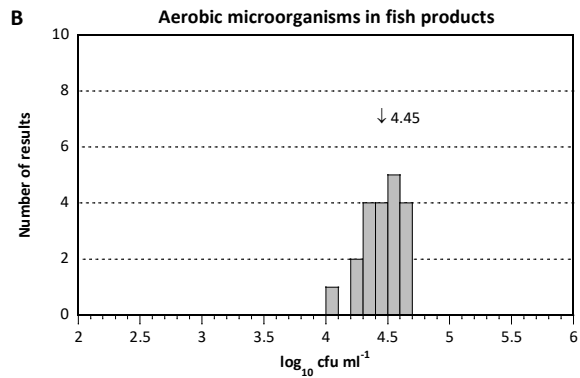
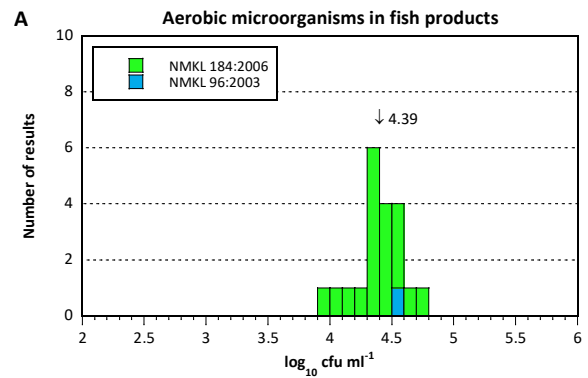
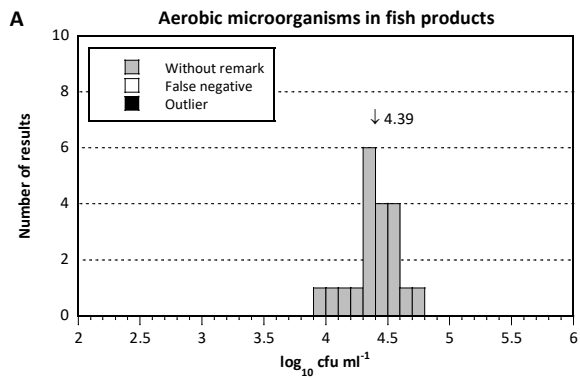
General remarks

Nineteen of the 20 participants 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 participants (95 %). One participant followed NMKL 96:2003, which is similar to NMKL 184:2006 when it comes to total aerobic count. The participant 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.

Table 11. Results from analysis of aerobic microorganisms in fish products, 20–25 °C.

Method	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	Med ¹	s	F	<	>
All results	20	20	4.39	0.20	0	0	0	20	20	4.45	0.16	0	0	0	20	18	4.12	0.14	0	1	1
NMKL 184:2006	19	19	4.38	0.20	0	0	0	19	19	4.44	0.16	0	0	0	19	17	4.13	0.14	0	1	1
NMKL 96:2003	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

¹ Median



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

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

No outliers or false negative results were reported.

Sample C

The strain of *S. putrefaciens* was target organism.

During quality control at the Swedish Food Agency the parameter did not fulfil the requirements for homogeneity, and a larger than usual distribution of the results could thus be expected. No values have therefore been considered as outliers. Participants that reported zero results are however encouraged to consider repeating the analysis.

The results for sample C are not evaluated, and no z-scores are calculated for the analysis.

General remarks

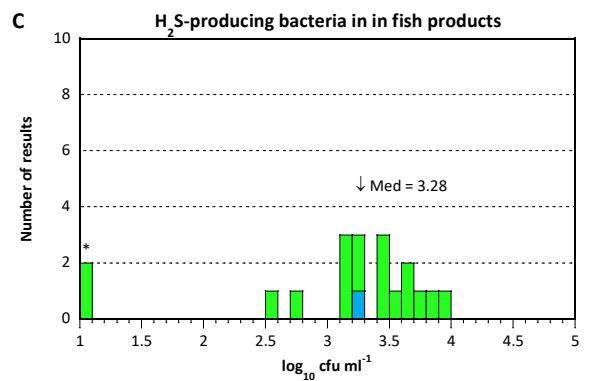
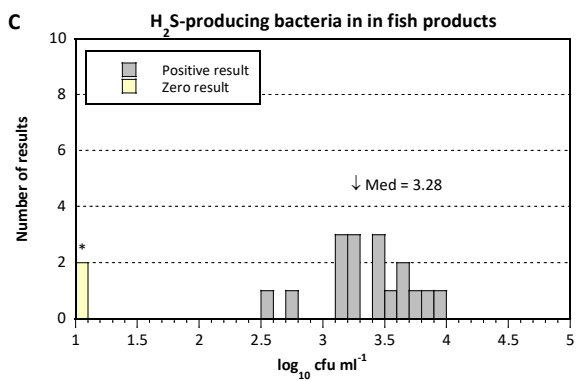
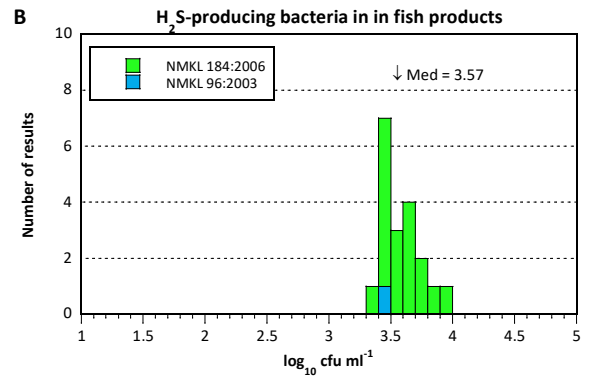
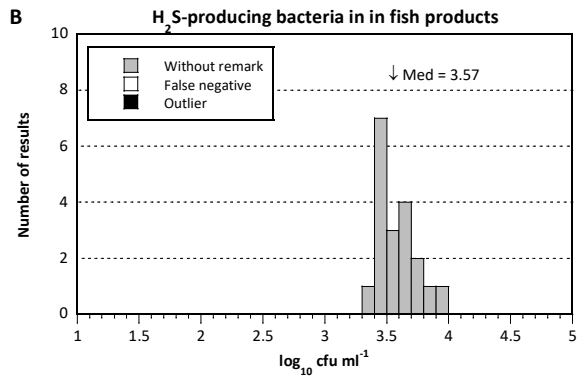
Nineteen of the 20 participants followed the method for aerobic microorganisms and specific spoilage organisms in fish and fish products, NMKL 184:2006. This prescribes a pour-plate method with IA, on which H₂S-producing bacteria form black colonies. One participant followed NMKL 96:2003 (“Bacterial examinations in fresh and frozen seafood”), which includes the analysis of H₂S-producing bacteria. This participant 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.

Table 12. Results from analysis of H₂S-producing bacteria in fish products.

Method	Sample A							Sample B							Sample C ¹						
	N	n	Med ²	s	F	<	>	N	n	Med ²	s	F	<	>	N	n	Med ²	s	F	<	>
All results	19	18	-	-	1	-	-	19	19	3.57	0.15	0	0	0	19	0	3.28	1.11	-	-	-
NMKL 184:2006	18	17	-	-	1	-	-	18	18	3.58	0.16	0	0	0	18	0	3.35	1.15	-	-	-
NMKL 96:2003	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	0	-	-	-	-	-

¹ The results for sample C are not evaluated

² Median



Yeasts and moulds

Sample A

The strain of *K. marxianus* was target organism for the analysis of yeasts, and the strain of *C. cladosporioides* was target organism for the analysis of moulds.

For yeasts, three low and three high outliers were reported, as well as three false negative results.

For moulds, one low and five high outliers were reported, as well as nine false negative results.

Five of the participants used TEMPO YM, which gives a combined result for yeasts and moulds. This is too few participants to perform a statistical analysis. The results for TEMPO YM are instead evaluated based on the sum of the assigned values for yeasts and moulds ($m_Y = 2.24$ and $m_M = 1.81 \log_{10} \text{ cfu ml}^{-1}$, respectively), and the pooled standard deviation of the method in the Swedish Food Agency's PT during 2017–2021 ($s_{\text{TEMPO}} = 0.31$).

For TEMPO YM, results between $m_{YM} \pm 2 s_{\text{TEMPO}}$ are considered acceptable, which corresponds to results between 1.77 and 2.99 $\log_{10} \text{ cfu ml}^{-1}$.

Sample B

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

For yeasts, two false positive result were reported.

For moulds, two false positive result were reported.

Sample C

The strain of *H. uvarum* was target organism for the analysis of yeasts. No target organism was present for the analysis of moulds.

For yeasts, six low outliers were reported.

For moulds, seven false positive results were reported.

General remarks

In essence, the same participants analysed both yeasts and moulds, and they generally used identical methods for both parameters. The methods mainly consisted of NMKL 98:2005, ISO 6611:2004 / IDF 94:2004 and 3M™ Petrifilm™, but ISO 21527-1:2008 / ISO 21527-2:2008 was also used by a few participants. One participant 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.

With NMKL 98:2005, participants mainly used either DRBC and/or DG18. With ISO 6611:2004 / IDF 94:2004, which describes the enumeration of yeasts and moulds in milk and milk products, participants instead mainly used YGC. ISO 21527-1:2008 and ISO 21527-2:2008 stipulate the use of DRBC and DG18, respectively. In general, DRBC is recommended for food with water activity $a_w > 0.95$ (e.g. fresh

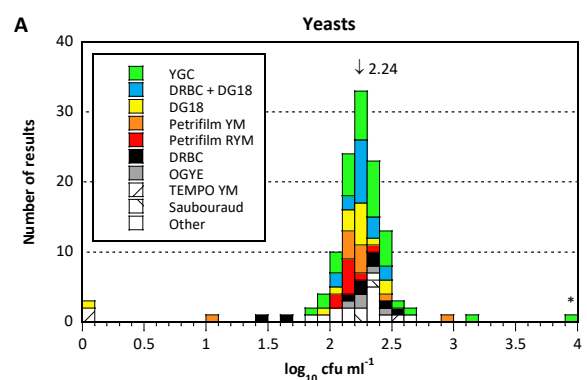
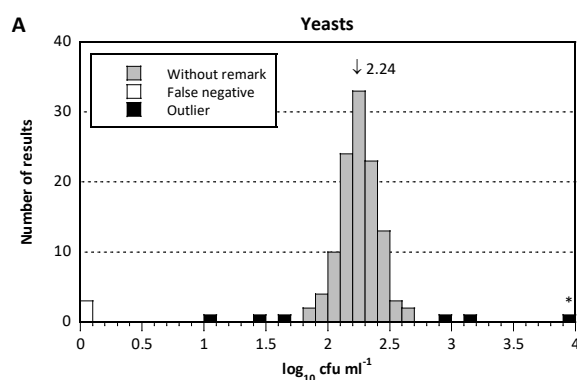
fruit, vegetables, meat and milk products) while DG18 is recommended for food with $a_w \leq 0.95$ (e.g. dried fruit, dried meat, grains and nuts).

Outliers and false results were for the most part evenly distributed between the main methods and media that were used. Though there could be a small over-representation of outliers and false results for moulds, by participants that used Petrifilm YM.

As discussed above, five participants used TEMPO YM, in one instance in combination with Petrifilm YM. The results from these five participants have for practical reasons still been included in the statistical analysis, and in tables and figures in this report. There, they may (inaccurately) appear as outliers and/or false results. **Results from TEMPO YM are however specifically – and only – evaluated according to the limits provided above for sample A.**

Table 13. 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	123	114	2.24	0.15	3	3	3	123	121	-	-	2	-	-	124	118	3.31	0.22	0	6	0
YGC	36	34	2.25	0.18	0	0	2	36	36	-	-	0	-	-	37	37	3.29	0.25	0	0	0
DRBC + DG18	18	18	2.25	0.10	0	0	0	18	18	-	-	0	-	-	18	18	3.40	0.20	0	0	0
DG18	15	14	2.22	0.12	1	0	0	15	14	-	-	1	-	-	15	12	3.35	0.18	0	3	0
Petrifilm YM	11	9	2.23	0.09	0	1	1	11	11	-	-	0	-	-	11	10	3.24	0.12	0	1	0
Petrifilm RYM	9	9	2.16	0.10	0	0	0	9	9	-	-	0	-	-	9	9	3.19	0.25	0	0	0
DRBC	9	7	2.31	0.14	0	2	0	9	9	-	-	0	-	-	9	7	3.41	0.26	0	2	0
OGYE	5	5	2.27	0.11	0	0	0	5	5	-	-	0	-	-	5	5	3.41	0.19	0	0	0
TEMPO YM	5	3	-	-	2	0	0	5	5	-	-	0	-	-	5	5	-	-	0	0	0
Saubouraud	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Other	12	12	-	-	0	0	0	12	11	-	-	1	-	-	12	12	-	-	0	0	0



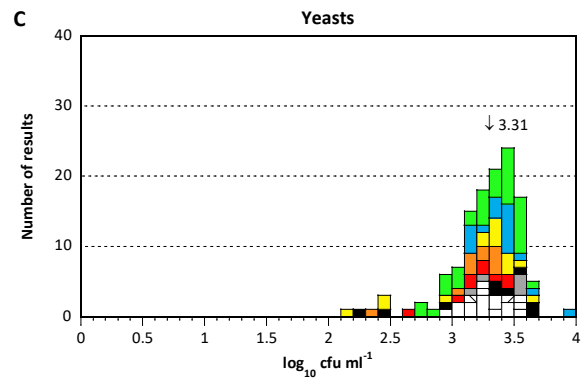
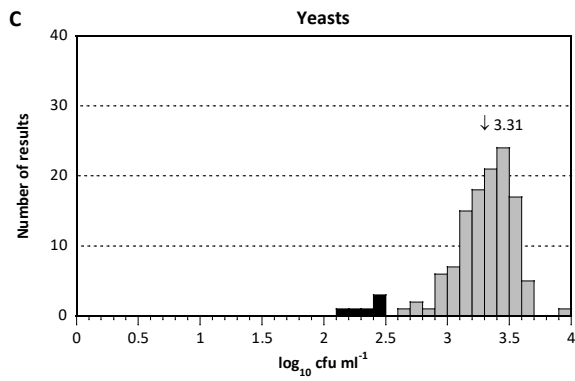
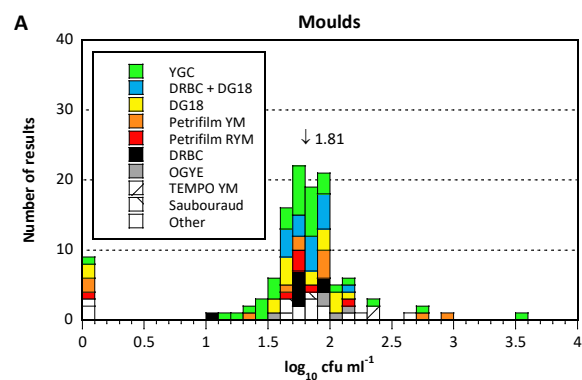
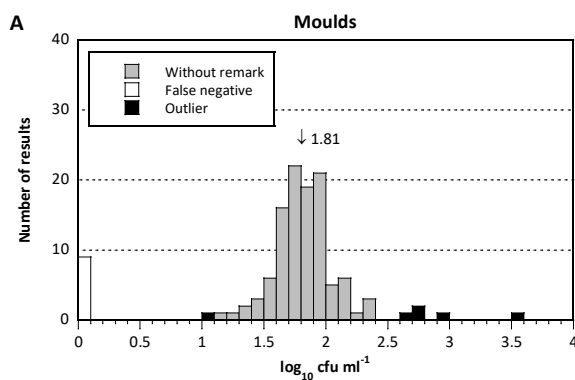


Table 13. Results from analysis of moulds.

Medium	Sample A							Sample B							Sample C						
	N	n	m	s	F	<	>	N	n	m	s	F	<	>	N	n	m	s	F	<	>
All results	121	106	1.81	0.21	9	1	5	121	119	-	-	2	-	-	120	113	-	-	7	-	-
YGC	35	32	1.72	0.25	1	0	2	35	34	-	-	1	-	-	35	35	-	-	0	-	-
DRBC + DG18	18	18	1.83	0.14	0	0	0	18	18	-	-	0	-	-	18	18	-	-	0	-	-
DG18	17	15	1.84	0.19	2	0	0	17	16	-	-	1	-	-	16	16	-	-	0	-	-
Petrifilm YM	12	8	1.76	0.22	2	0	2	12	12	-	-	0	-	-	12	9	-	-	3	-	-
Petrifilm RYM	7	6	1.81	0.16	1	0	0	7	7	-	-	0	-	-	7	6	-	-	1	-	-
DRBC	8	7	1.80	0.10	0	1	0	8	8	-	-	0	-	-	8	8	-	-	0	-	-
OGYE	5	5	1.92	0.21	0	0	0	5	5	-	-	0	-	-	5	5	-	-	0	-	-
TEMPO YM	4	2	-	-	1	0	1	4	4	-	-	0	-	-	4	1	-	-	3	-	-
Saubouraud	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	-	-
Other	12	10	-	-	2	0	0	12	12	-	-	0	-	-	12	12	-	-	0	-	-



Outcome of the results of individual participants - assessment

Reporting and evaluation of results

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

Participants are not grouped or ranked based on their results. The performance of an individual participant can be broadly assessed by the numbers of outliers and false results, and by the z-scores.

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol [4].

Samples for follow-up analyses can be ordered at: www.livsmedelsverket.se/en/PT-extra

Box plots and numbers of deviating results for each participant

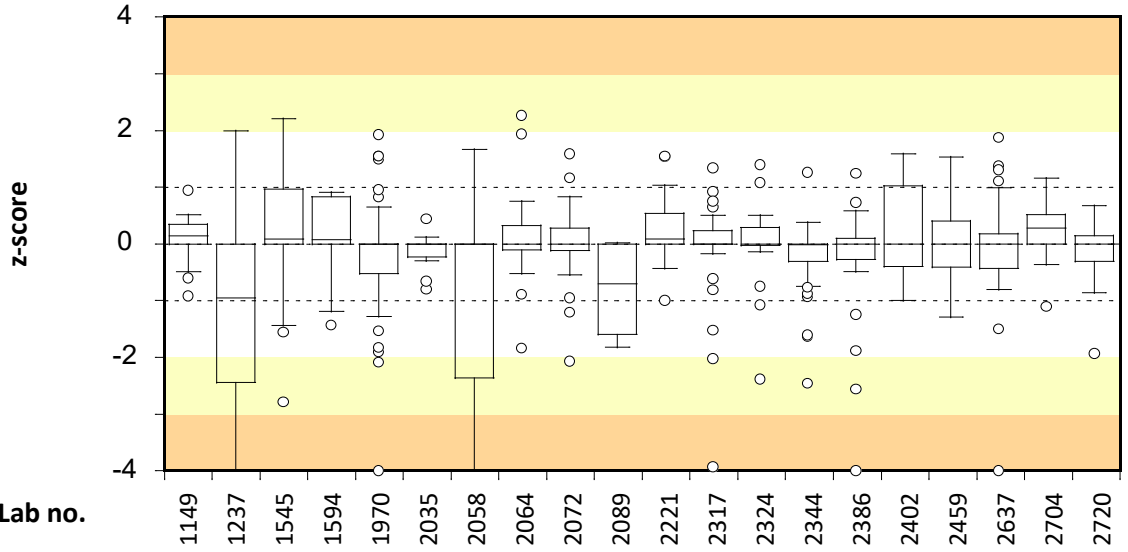
Box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the performance of each participant. The range of z-scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred around zero, indicates that the results of the individual participant are in general close to m_{PT} for the different analyses. For each participant, the number of false results and outliers are also listed in the tables below the box plots.

Outliers are included in the figures after being calculated to z-scores in the same way as for other results. Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism are given a z-score of 0. False results do not generate any z-scores, and are not included in “No. of results”.

The participant’s median value is illustrated by a horizontal line in the box. Each box includes 50 % of a participant’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.

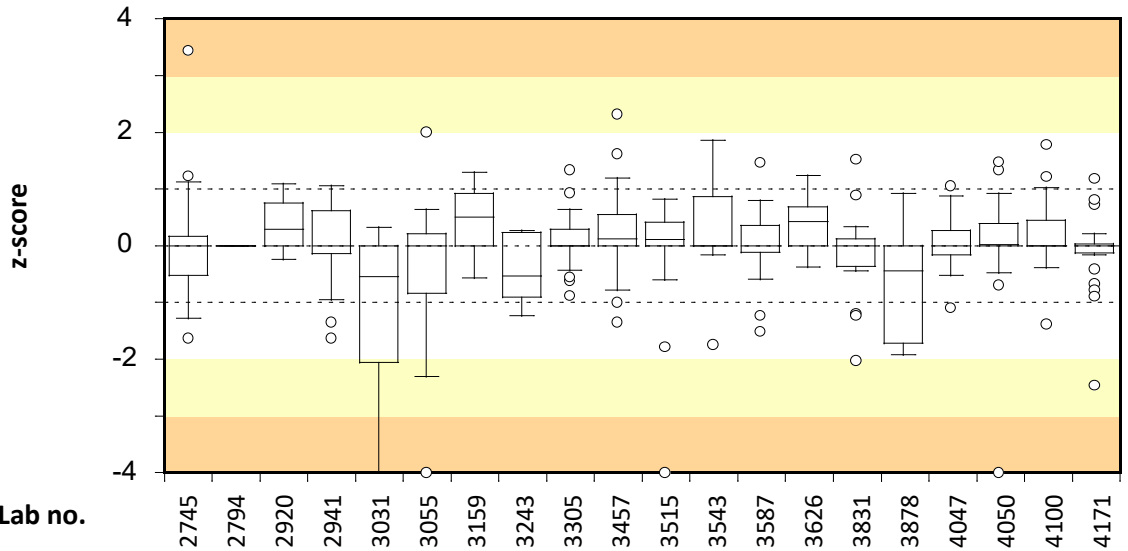
Z-scores $>+4$ and <-4 are positioned at $+4$ and -4 , respectively, in the plot. The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

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



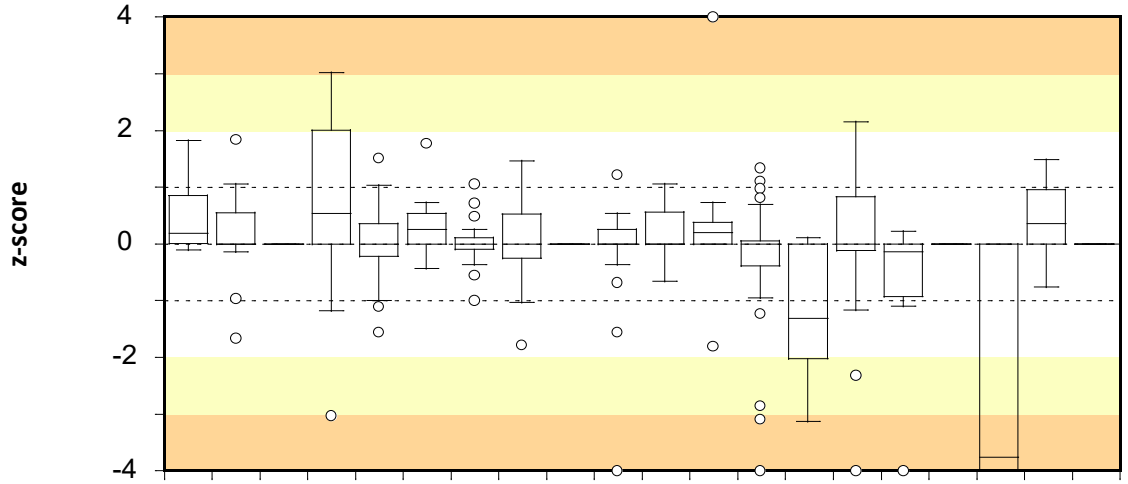
Lab no.

No. of results	14	29	26	18	37	12	19	15	32	7	28	26	18	29	20	12	21	29	23	15	
False positive	1	3	1	-	-	-	1	-	-	1	1	1	-	-	-	-	-	-	-	-	-
False negative	-	1	-	-	-	-	1	-	-	1	1	-	1	-	1	-	-	-	1	-	-
Low outliers	-	2	-	-	1	-	3	-	-	-	-	1	-	-	1	-	-	-	1	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

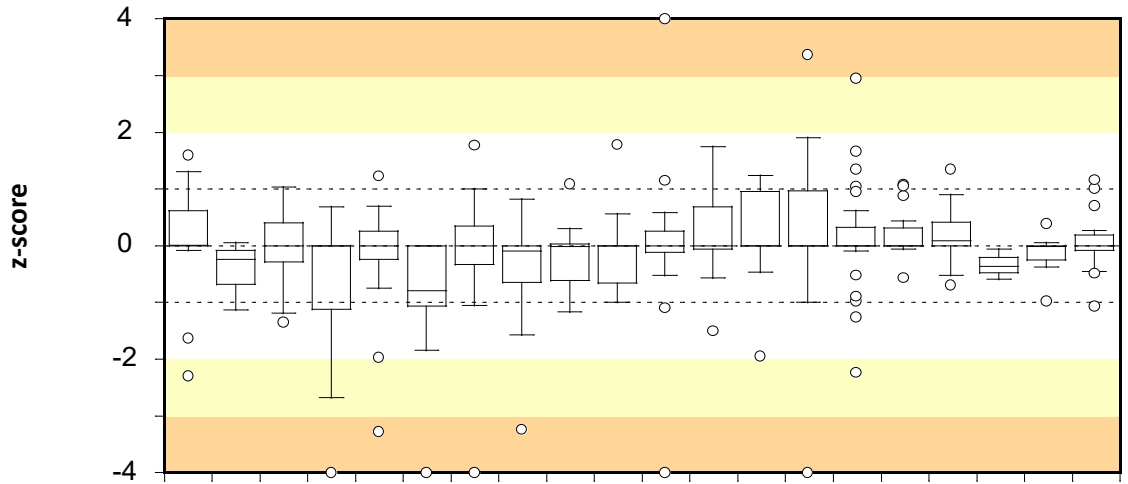


Lab no.

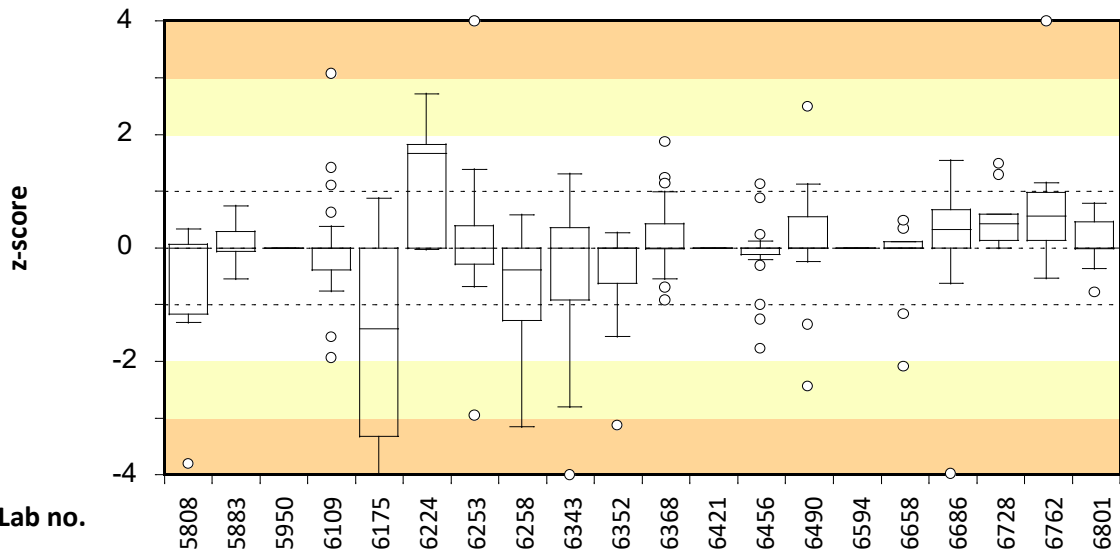
No. of results	25	-	11	26	11	14	12	6	35	24	15	17	23	18	15	20	21	18	31	25	
False positive	1	-	-	-	-	1	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-
False negative	1	-	1	1	-	-	-	-	-	2	-	1	1	-	-	1	-	-	-	1	-
Low outliers	-	-	-	-	2	1	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-
High outliers	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



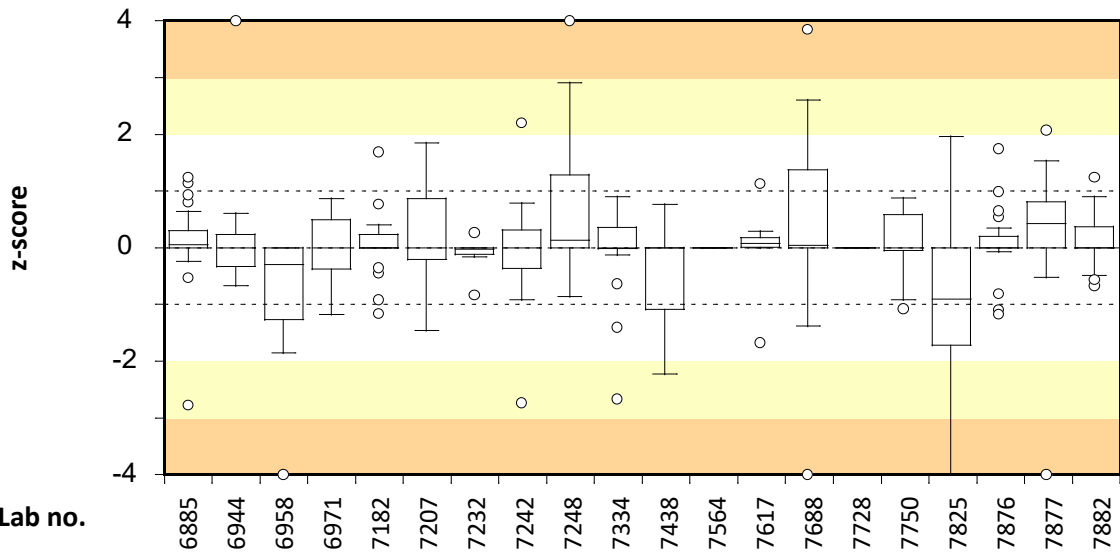
Lab no.	4246	4288	4339	4400	4449	4557	4635	4664	4683	4817	4878	4889	4944	4951	4980	4983	5018	5100	5119	5128
No. of results	11	25	-	17	15	11	18	22	-	21	15	23	32	15	32	13	-	9	12	-
False positive	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
False negative	1	1	-	1	-	1	1	1	-	-	-	-	-	-	-	1	-	-	2	-
Low outliers	-	-	-	-	-	-	-	-	-	1	-	-	1	-	1	1	-	5	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-



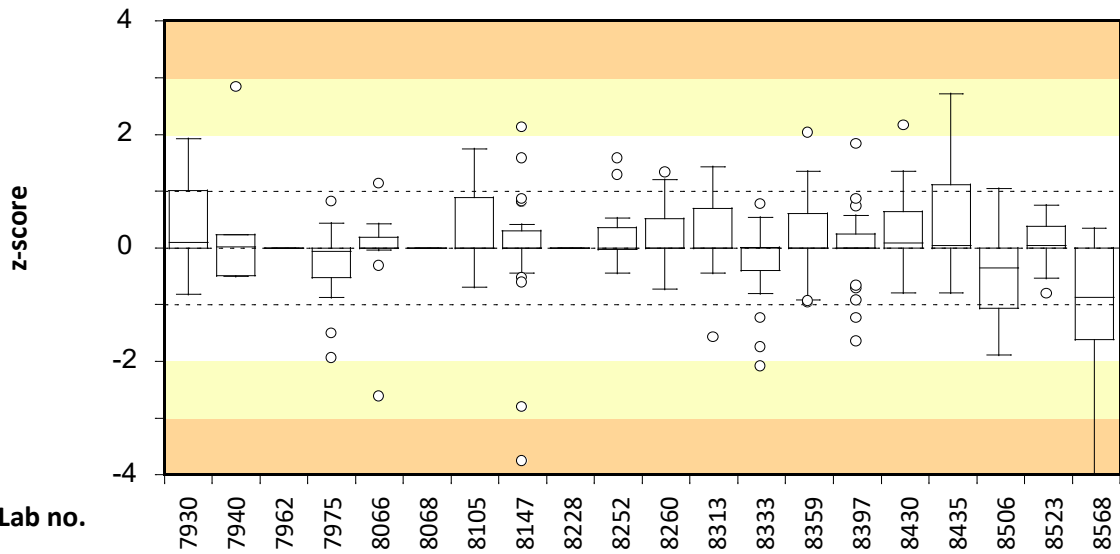
Lab no.	5182	5188	5200	5201	5204	5220	5261	5329	5338	5352	5419	5545	5553	5612	5615	5632	5654	5701	5774	5801
No. of results	18	7	21	21	32	24	16	23	12	24	25	18	18	21	26	15	15	3	11	15
False positive	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	1	-
False negative	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-	-	-
Low outliers	-	-	-	2	-	2	1	-	-	-	1	-	-	1	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-



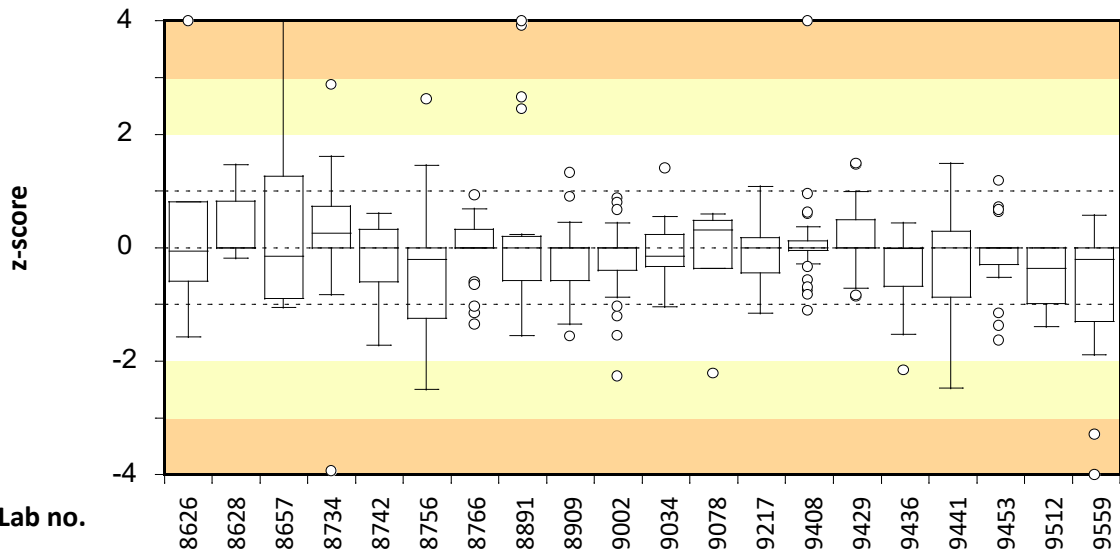
Lab no.	5808	5883	5950	6109	6175	6224	6253	6258	6343	6352	6368	6421	6456	6490	6594	6658	6686	6728	6762	6801
No. of results	8	30	-	21	10	9	18	9	28	25	31	-	21	19	-	9	27	10	8	15
False positive	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	1	-	-	-	-
False negative	1	-	-	-	1	-	-	2	-	2	-	-	-	2	-	-	1	2	1	-
Low outliers	1	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-
High outliers	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-



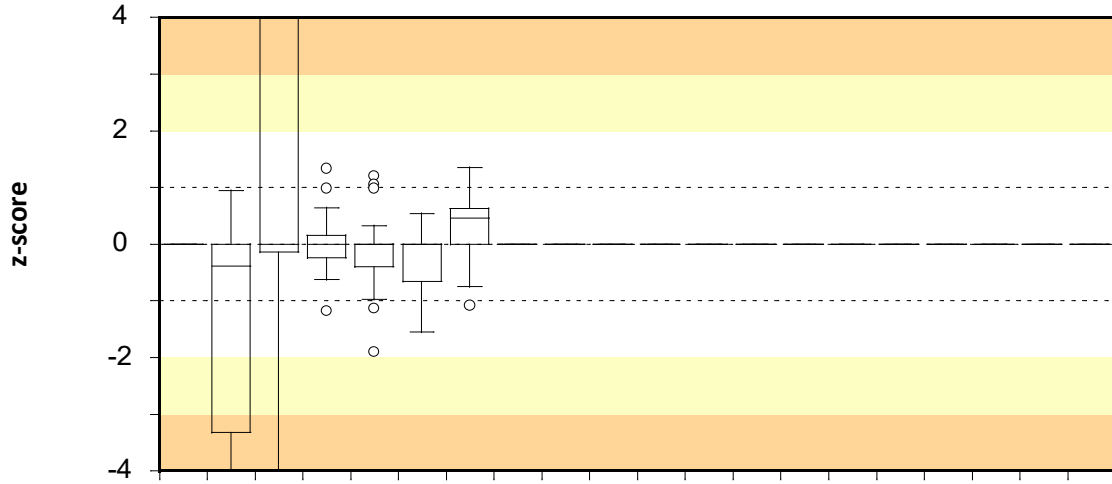
Lab no.	6885	6944	6958	6971	7182	7207	7232	7242	7248	7334	7438	7564	7617	7688	7728	7750	7825	7876	7877	7882
No. of results	24	16	15	9	17	16	9	12	33	19	26	-	11	31	-	12	18	24	14	28
False positive	-	1	-	-	1	2	-	-	3	-	-	-	-	1	-	-	2	-	-	1
False negative	-	1	-	-	-	-	-	-	1	-	1	-	1	-	-	-	1	-	-	1
Low outliers	-	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-	1	-
High outliers	-	1	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-	-



Lab no.	7930	7940	7962	7975	8066	8068	8105	8147	8228	8252	8260	8313	8333	8359	8397	8430	8435	8506	8523	8568
No. of results	29	6	-	14	18	-	18	29	-	15	27	18	24	24	22	17	33	15	16	24
False positive	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	1	2	-	-
False negative	-	-	-	1	1	-	-	1	-	-	-	-	-	-	1	1	1	1	2	-
Low outliers	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	3
High outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	8626	8628	8657	8734	8742	8756	8766	8891	8909	9002	9034	9078	9217	9408	9429	9436	9441	9453	9512	9559
No. of results	9	35	10	13	9	19	24	20	17	29	11	6	17	31	27	31	32	17	14	21
False positive	-	-	-	2	-	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-
False negative	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	1	-	1	1	-
Low outliers	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
High outliers	2	-	1	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-



Lab no.	9662	9747	9783	9886	9890	9903	9950
No. of results	-	14	10	30	23	24	14
False positive	-	1	2	1	-	-	1
False negative	-	-	-	1	1	-	-
Low outliers	-	3	1	-	-	-	-
High outliers	-	-	3	-	-	-	-

Test material and quality control

Test material

Each participant received three samples with freeze-dried microorganisms, designated A–C. The test material was freeze-dried in 0.5 ml portions in glass vials, as described by Peterz and Steneryd [5]. Before analysing the samples, the contents of each vial should be reconstituted in 254 ml of sterile diluent. The microorganism content of the samples and the concentrations determined at the Swedish Food Agency are listed in the table below.

Table 14. Microorganisms in the samples.

Sample	Microorganism	Strain			
		SLV no. ¹	Origin	Reference ²	log ₁₀ cfu ml ⁻¹
A	<i>Bacillus cereus</i>	SLV-160	-	CCUG 45098	4.57
	<i>Cladosporium cladosporioides</i>	SLV-488	meat stamp	CBS 812.96	1.99
	<i>Escherichia coli</i>	SLV-524	chicken	CCUG 47554	3.76
	<i>Kluyveromyces marxianus</i>	SLV-439	-	-	2.39
	<i>Lactobacillus plantarum</i>	SLV-475	-	CCUG 30503	3.67
	<i>Staphylococcus xylosum</i>	SLV-283	cheese	-	3.25
B	<i>Aeromonas hydrophila</i>	SLV-467	drinking water pipe	CCUG 46535	N/A
	<i>Clostridium bifermentans</i>	SLV-009	fish	CCUG 43592	3.24
	<i>Escherichia coli</i>	SLV-082	drinking water	CCUG 45097	4.19
	<i>Hafnia alvei</i>	SLV-015	minced meat	CCUG 45642	3.30
	<i>Lactobacillus plantarum</i>	SLV-445	-	ATCC 8014	4.30
	<i>Staphylococcus aureus</i>	SLV-350	-	CCUG 45099	3.55
C	<i>Clostridium perfringens</i>	SLV-442	-	CCUG 43593	2.90
	<i>Hanseniaspora uvarum</i>	SLV-555	-	-	3.43
	<i>Serratia marcescens</i>	SLV-040	pond water	-	2.77
	<i>Shewanella putrefaciens</i>	SLV-520	-	CCUG 46538	3.57
	<i>Staphylococcus aureus</i>	SLV-539	mastitis	-	4.14

¹ Internal strain identification no. at the Swedish Food Agency

² Culture collection. ATCC: American Type Culture Collection, CBS: Centraalbureau voor Schimmelcultures (Westerdijk Institute), CCUG: Culture Collection University of Gothenburg, Sweden; SMI: Public Health Agency of Sweden.

Quality control of the samples

In order to allow comparison of the freeze-dried samples, it is essential to have aliquots of homogeneous test material 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 test material is approved if, for each analysis, the values obtained for the test for “Index of dispersion” between vials (I_2) and the test for reproducibility (T) do not simultaneously exceed 2.0 and 2.6, respectively. (For definitions of I_2 , and T, see references [6] and [7] respectively.)

Table 15. 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 ¹			C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.61	0.89	1.36	4.65	0.81	1.29	4.21	0.35	1.52
Psychrotrophic microorganisms NMKL method no. 86:2013	-	-	-	3.68	0.76	1.28	3.34	0.98	4.26
Enterobacteriaceae NMKL method no. 144:2005	3.64	0.33	1.19	4.24	1.03	1.25	2.77	1.41	1.37
<i>Escherichia coli</i> NMKL method no. 125:2005	3.76	1.75	1.41	4.19	2.41	1.41	-	-	-
Presumptive <i>Bacillus cereus</i> NMKL method no. 67:2010	4.57	0.49	1.26	-	-	-	-	-	-
Coagulase-positive staphylococci NMKL method no. 66:2009	-	-	-	3.55	0.33	1.21	4.14	5.28	1.73
Lactic acid bacteria NMKL method no. 140:2007	3.67	0.31	1.17	4.30	1.16	1.23	-	-	-
<i>Clostridium perfringens</i> NMKL method no. 95:2009	-	-	-	-	-	-	2.76	1.67	1.42
Anaerobic sulphite-reducing bacteria NMKL method no. 56:2015	-	-	-	3.24	2.59	1.40	2.90	0.33	1.14
Aerobic microorganisms in fish products NMKL method no. 184:2006	4.58	0.49	1.27	4.59	0.45	1.25	3.70	8.40	2.41
H ₂ S-producing bacteria in fish products NMKL method no. 184:2006	-	-	-	3.30	0.86	3.99	3.57	7.21	2.61
Yeasts NMKL method no. 98:2005 (DRBC)	2.39	2.21	1.76	-	-	-	3.43	0.79	1.40
Moulds NMKL method no. 98:2005 (DRBC)	1.99	1.20	1.95	-	-	-	-	-	-

– No target organism or no value

¹ n = 5 vials analysed in duplicate

References

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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.
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6. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockefeer, 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.
7. 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.

Table with 17 columns for Lab no and 16 columns for Vial, and a grid of numerical data for various microorganism types (Aerobic, Psychrotrophic, Enterobacteriaceae, Escherichia coli, Presumptive Bacillus cereus, etc.) and a final Lab no column.

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, 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							
9886	3	2	1	4.54	4.6	4.01	4.39	3.73	3.56	3.52	4.06	2.62	3.48	4	<1	4.2	<1	<1	<1	3.57	3.98	3.63	4.27	<1	<1	3.3	2.78	<1	3.23	<1	-	-	-	-	-	-	2.15	<1	3.53	1.72	<1	<1	9886	
9890	2	1	3	4.66	4.52	4.04	-	-	-	3.54	3.86	0	3.48	3.88	0	4.38	0	0	0	3.34	3.71	3.65	4.18	0	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4	0	3.53	1.85	0	0	9890
9903	2	3	1	4.25	4.45	3.99	-	-	-	3.56	3.89	2.55	3.66	4.02	<0	4.22	<1	<1	<0	3.49	3.75	-	-	-	<0	<0	2.62	-	-	-	-	-	-	-	-	-	-	2.26	<0	3.4	1.67	<0	<0	9903
9950	2	3	1	4.53	4.45	4.13	-	-	-	3.71	4.32	2.76	-	-	-	-	-	-	-	-	-	3.67	4.31	1.58	-	-	-	-	-	-	-	-	-	-	-	-	-	2.15	0	3.15	1.94	0	0	9950
N				147	148	148	15	15	15	126	127	127	103	103	104	108	108	108	95	94	94	49	49	48	47	47	47	57	55	55	20	20	20	19	19	19	123	123	124	121	121	120	N	
Min				3.44	3.30	3.18	0	0	0	2.94	0	0	0	2.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.90	4.00	2.85	0	3.30	0	0	0	2.10	0	0	0	Min	
Max				4.76	4.95	5.75	4.64	4.74	4.86	4.00	4.60	4.46	5.38	5.38	3.76	4.85	3.97	3.70	2.81	4.41	4.32	3.85	4.57	5.56	0	3.43	3.20	0	3.90	3.30	4.70	4.65	5.46	1.00	3.93	3.95	4.70	2.91	3.95	3.59	3.79	3.48	Max	
Med				4.43	4.59	4.08	4.20	3.72	3.56	3.60	4.08	2.66	3.62	4.02	0	4.38	0	0	0	3.44	3.94	3.61	4.24	0	0	0	2.80	0	3.19	2.75	4.41	4.46	4.12	0	3.57	3.28	2.25	0	3.34	1.82	0	0	Med	
m_{PT}				4.405	4.587	4.076	3.619	3.835	3.685	3.597	4.069	2.650	3.547	3.971	0	4.394	0	0	0	3.437	3.930	3.611	4.243	0	0	0	2.716	0	3.184	2.659	4.391	4.451	4.098	0	3.573	3.010	2.243	0	3.313	1.805	0	0	m_{PT}	
s_{PT}				0.212	0.128	0.127	1.514	0.437	0.476	0.151	0.185	0.172	0.281	0.212	0	0.165	0	0	0	0.100	0.116	0.117	0.106	0	0	0	0.271	0	0.279	0.342	0.200	0.163	0.137	0	0.153	1.117	0.148	0	0.218	0.214	0	0	s_{PT}	
u_{PT}				0.018	0.011	0.011	0.391	0.117	0.132	0.014	0.017	0.016	0.029	0.021	0	0.017	0	0	0.000	0.011	0.013	0.017	0.015	0	0	0	0.042	0	0.038	0.047	0.045	0.036	0.032	0	0.035	0.256	0.014	0	0.020	0.021	0	0	u_{PT}	
F+				0	0	0	0	0	0	0	0	0	0	0	2	0	7	3	2	0	0	0	0	11	0	13	0	0	0	0	0	0	0	1	0	0	0	2	0	0	2	7	F+	
F-				0	0	0	0	1	2	0	1	14	7	0	0	4	0	0	0	1	14	1	0	0	0	0	3	0	1	2	0	0	0	0	3	0	0	9	0	0	F-			
<				1	4	2	0	0	0	1	2	2	1	4	0	9	0	0	0	5	1	1	2	0	0	0	2	0	1	0	0	0	1	0	0	0	6	1	0	0	<			
>				0	0	3	0	0	0	0	0	2	2	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	5	0	0	>			
< OK				3.70	4.20	3.73	0	3.17	3.11	3.10	3.46	2.17	2.68	3.30	0	3.97	0	0	0	3.11	3.64	3.21	4.05	0	0	0	2.01	0	2.41	1.65	3.90	4.00	3.76	0	3.30	0	1.87	0	2.63	1.13	0	0	< OK	
> OK				4.76	4.95	4.45	4.64	4.74	4.86	4.00	4.60	3.17	4.11	4.38	0	4.85	0	0	0	3.64	4.32	3.85	4.57	0	0	0	3.20	0	3.91	3.30	4.70	4.65	4.32	0	3.93	3.95	2.69	0	3.95	2.37	0	0	> OK	

N = number of analyses performed Max = highest reported result m = mean value F+ = false positive < = low outlier < OK = lowest accepted value u_(m) = 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 parameter is not evaluated
- Outlier, false positive or false negative
- Results "larger than" are not evaluated

Lab no.	Vial			Aerobic microorganisms 30 °C			Psychro-trophic microorg.			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 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				
9453	2	1	3	-1.624	-1.151	-0.522				-1.366		-0.290				-0.148	0	0	0	0.636	-0.256													0.720	0	0.672	1.189	0	0	9453			
9512	1	2	3	-0.984	-0.749	-0.550				-0.348	-0.385	-0.278																							-1.385	0	-1.006		0	0	9512		
9559	3	1	2	-0.211	-1.777	0.110				-4.000	-3.286	0.059	0.580	-0.619	0	-1.297	0	0	0	-1.370	-1.033													-0.896	0	-1.891	-0.630	0	0	9559			
9662	1	3	2																																					9662			
9747	3	2	1	-3.318	-4.000	-1.391				0.947	0.382	0.816				-4.000		0																	-0.762	0	-4.000	-0.770	0	0	9747		
9783	2	3	1	-0.136	-0.658	4.000										-4.000		0																	4.000	0	0.370	4.000	0	0	9783		
9886	3	2	1	0.636	0.101	-0.522	-0.240	-0.262		-0.507	-0.050	-0.174	-0.240	0.137	0	-1.176	0	0	0	1.338	0.434	0.160	0.251	0	0	0.235	0	0.165				-0.627	0	0.992	-0.397	0	0	9886					
9890	2	1	3	1.201	-0.525	-0.285				-0.375	-1.129		-0.240	-0.430	0	-0.087	0	0	0	-0.969	-1.895	0.331	-0.598	0									1.057	0	0.992	0.209	0	0	9890				
9903	2	3	1	-0.729	-1.073	-0.680				-0.243	-0.967	-0.581	0.402	0.232	0	-1.055	0	0	0	0.536	-1.550				0	0	-0.356						0.114	0	0.397	-0.630	0	0	9903				
9950	2	3	1	0.589	-1.073	0.426				0.749	1.352	0.641																						-0.627	0	-0.747	0.629	0	0	9950			

 The analysis is 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 the analytical work also needs 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 PT, identical test material is analysed by a number of participants. After reporting of results by the participants, 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 samples 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