Proficiency testing Food Microbiology

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Contents

Abbreviations

Media

ALOA	Agar for Listeria according to Ottaviani & Agosti
APW 2%	Alcaline peptone water, 2 % NaCl
BA	Blood agar
BcsA	*Bacillus cereus* 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™ *Bacillus cereus* agar
CIN	Cefsulodin irgasan novobiocin agar
Compact Dry EC	Compact Dry™ *E. coli* 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	*E. coli* broth
ENT	Slanetz & Bartley *Enterococcus* agar
HEA	Hektoen enteric agar
IA	Iron agar
ISA	Iron sulphite agar
ITC	Irgasan ticarcillin potassium chlorate broth
KEAA	Kanamycin esculin azide agar
LMBA	*Listeria monocytogenes* blood agar
LSB	Lauryl sulphate broth
LTLSB	Lactose tryptone lauryl sulphate broth
mCCDA	Modified charcoal cephoperazone deoxycholate agar
mCP	Membrane *Clostridium perfringens* 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
NAP	Nitrite actidione 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™ *E. coli*/Coliform count
Petrifilm EL	3M™ Petrifilm™ Environmental Listeria
Petrifilm LAB	3M™ Petrifilm™ Lactic acid bacteria
Petrifilm RAC	3M™ Petrifilm™ Rapid Aerobic Count
Petrifilm REC	3M™ Petrifilm™ Rapid *E. coli*/Coliform count
Petrifilm SEC	3M™ Petrifilm™ Select *E. coli*
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® *Bacillus cereus*
TEMPO CAM	TEMPO® Campylobacter
TEMPO CC	TEMPO® Coliform count
TEMPO EB	TEMPO® Enterobacteriaceae
TEMPO EC	TEMPO® E. coli
TEMPO RYM	TEMPO® Rapid Yeast/Mould
TEMPO STA	TEMPO® Coagulase-positive staphylocci
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
ATCC	American Type Culture Collection
CBS	Centraalbureau voor Schimmelcultures (Westerdijk Institute)
CCUG	Culture Collection University of Gothenburg
IDF	International Dairy Foundation
ISO	International Organization for Standardization
NMKL	Nordic-Baltic Committee on Food Analyses
NordVal	NordVal International - NMKL
SLV	Livsmedelsverket/Swedish Food Agency, Sweden
Fohm	Public Health Agency of Sweden

Analyses in this PT round

Quantitative analyses

Aerobic microorganisms, 30 °C

Aerobic microorganisms, 20 °C

Contaminating microorganisms in dairy products

Enterobacteriaceae

Coliform bacteria 30 °C

Coliform bacteria 37 °C

Thermotolerant coliform bacteria

Escherichia coli

Presumptive *Bacillus cereus*

Coagulase-positive staphylococci

Enterococci

Gram-negative bacteria in pasteurised milk and cream

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.

It is also 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.

Standard deviation and assigned value

Evaluation of the participants' results and statistical calculations are carried out on the $\log_{10}$ transformed results. Results reported by participants as "> value" are not evaluated. Results reported as "< value" are treated as zero (negative result).

A robust statistical approach is used to determine the mean value and standard deviation. Algorithm A with iterated scale as described in ISO 13528:2022 [1] is used to determine the robust mean (m_{PT}) and robust standard deviation (s_{PT}) of the participants' results. Results that are obviously erroneous are excluded prior to determining m_{PT} and s_{PT} (blunder removal). For evaluated parameters, the assigned value consists of m_{PT}. It is regarded as the true, normative value.

For small datasets, there is an increased uncertainty associated with determining the robust mean (m_{PT}) and robust standard deviation (s_{PT}) of the participants' results. Therefore, when fewer than 12 participants have reported evaluated results, the statistical measures for performance evaluation will be provided *only as an information* to the participants.

Outliers

Outliers are results that deviate from the other results in a way that cannot be explained by normal variation. Results within $m_{PT} \pm 3s_{PT}$ are considered acceptable, whereas results outside this interval are considered as outliers. When fewer than 12 participants have reported results, as well as in some individual cases, subjective adjustments are made to set acceptance limits based on prior knowledge of the samples contents.

Results from different methods

Non-robust median values (*Med*) and standard deviations (*s*) are calculated to assist in the evaluation of the results from different methods. These are shown in tables in the report, in connection with the respective analyses. In these instances, *Med* and *s* are calculated from the respective method groups' results, with outliers and false results excluded. For method groups with fewer than 5 results, only the number of false results and outliers are provided.

Measurement uncertainty for the assigned values

The standard uncertainty (u_{PT}) of the assigned value (m_{PT}) is estimated from the standard deviation (s_{PT}) and the number of evaluated results (n):

$$u_{PT} = 1.25 \times \frac{s_{PT}}{\sqrt{n}}$$

The measurement uncertainty is considered negligible compared to the standard deviation (which is used for evaluating the participants' results) when:

$$u_{PT} < 0.3 s_{PT}$$

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, robust mean value in $\log_{10}$ cfu ml^{-1}
s_{PT}	robust standard deviation
u_{PT}	standard uncertainty of the assigned value

F	number of false positive or false negative results
<	number of low outliers
>	number of high outliers
▭	results deviating more than 1 s_{PT} 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

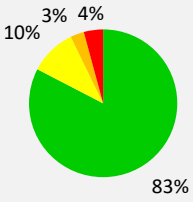
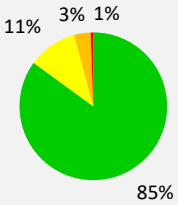
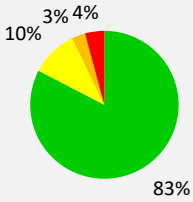
Results

General outcome

Samples were sent to 174 participants; 42 in Sweden, 115 in Europe, and 17 outside of Europe. In total, 167 participants (96 %) reported results, of which 45 (27 %) provided at least one result that received a remark.

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 remarks 1 remark 2 remarks >2 remarks												
Microorganisms	*Bacillus cereus* *Escherichia coli* *Enterococcus durans* *Staphylococcus aureus*				*Bacillus cereus* *Enterococcus durans* *Staphylococcus xylosus*				*Bacillus cereus* *Escherichia coli* *Enterococcus durans* *Staphylococcus aureus*			
Analysis	**Target organism**	**N**	**F**	**X**	**Target organism**	**N**	**F**	**X**	**Target organism**	**N**	**F**	**X**
Aerobic micro-organisms, 30 °C	**All**	154	0	8	**All**	154	0	11	**All**	154	0	10
Aerobic micro-organisms, 20 °C	**All**	26	0	1	**All**	27	0	2	**All**	28	0	1
Contaminating microorganisms	**All**	15	0	0	**All**	16	1	0	**All**	15	0	0
Enterobacteriaceae	***E. coli***	138	0	8	-	139	0	0	***E. coli***	138	0	6
Coliform bacteria, 30 °C	***E. coli***	38	2	0	-	40	0	0	***E. coli***	40	2	1
Coliform bacteria, 37 °C	***E. coli***	85	2	2	-	86	0	0	***E. coli***	86	1	3
Thermotolerant coliform bacteria	***E. coli***	36	0	0	-	38	0	0	***E. coli***	37	0	0
Escherichia coli	***E. coli***	106	0	6	-	109	0	0	***E. coli***	107	0	6
Presumptive *Bacillus cereus*	***B. cereus***	102	0	5	***B. cereus***	102	5	5	***B. cereus***	103	0	7
Coagulase-positive staphylococci	***S. aureus***	89	3	8	*(S. xylosus)*	89	6	0	***S. aureus***	90	3	8
Enterococci	***E. durans***	54	3	3	***E. durans***	55	0	5	***E. durans***	55	1	3
Gram-negative bacteria	***E. coli***	11	0	0	-	11	0	0	***E. coli***	11	0	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 and 20 °C

Sample A

All strains in the sample were target organisms. They were present in similar concentrations.

For the analysis at 30 °C, 154 participants reported results. Five low and three high outliers were reported.

For the analysis at 20 °C, 26 participants reported results. One low outlier was reported.

Sample B

All strains in the sample were target organisms. *S. xylosus* was present in a higher concentration than *B. cereus* and *E. durans*.

For the analysis at 30 °C, 154 participants reported results. Eight low and three high outliers were reported.

For the analysis at 20 °C, 27 participants reported results. Two low outliers were reported.

Sample C

The sample was identical to sample A.

For the analysis at 30 °C, 154 participants reported results. Six low and four high outliers were reported.

For the analysis at 20 °C, 28 participants reported results. One low outlier was reported.

General remarks

Most participants followed either NMKL 86:2013, ISO 4833-1:2013 or used 3M Petrifilm AC. At 30 °C, the withdrawn NMKL 86:2006 and ISO 4833:2003 were still used by ten and six 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 incubation 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). NMKL 86:2013 was last reviewed by NMKL in 2022 and remains current.

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. For sample A, the mean value from participants that used TEMPO AC was somewhat higher compared to participants that used other methods. Differences of this magnitude are not uncommon for TEMPO methods, and could be considered normal.

At 20 °C, IA was used by participants that followed NMKL 184. This method is adapted for aerobic microorganisms and specific spoilage microorganisms in fish and fish products.

At 30 °C, two participants followed ISO 13559/IDF 153, which is adapted for the enumeration of contaminating microorganisms. Also at 30 °C, one participant followed IDF 100B:1991. This method has been withdrawn, and replaced by ISO 4833.

Overall, the results for the identical samples A and C were highly similar.

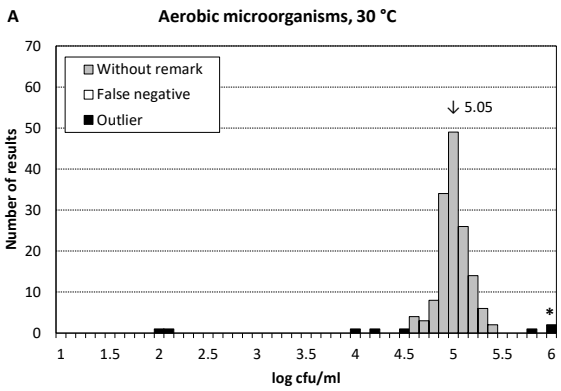
Table 2. Results from analysis of aerobic microorganisms, 30 °C.

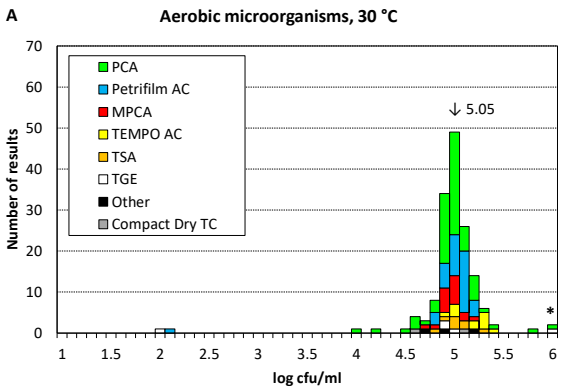
Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**154**	**146**	**5.05**	**0.15**	**0**	**5**	**3**	**154**	**143**	**5.11**	**0.13**	**0**	**8**	**3**	**154**	**144**	**5.04**	**0.13**	**0**	**6**	**4**
PCA	68	63	5.01	0.15	0	3	2	68	63	5.14	0.12	0	4	1	68	64	5.02	0.13	0	2	2
Petrifilm AC[1]	39	38	5.10	0.11	0	1	0	39	37	5.13	0.12	0	2	0	39	37	5.08	0.13	0	2	0
MPCA	18	18	5.01	0.12	0	0	0	18	18	5.05	0.13	0	0	0	18	18	5.02	0.10	0	0	0
TEMPO AC	11	11	5.26	0.17	0	0	0	11	10	5.13	0.14	0	0	1	11	10	5.15	0.05	0	0	1
TSA	8	8	5.07	0.14	0	0	0	8	8	5.17	0.14	0	0	0	8	8	5.07	0.10	0	0	0
TGE	5	3	-	-	0	1	1	5	3	-	-	0	1	1	5	3	-	-	0	1	1
Other	3	3	-	-	0	0	0	3	2	-	-	0	1	0	3	2	-	-	0	1	0
Compact Dry TC[2]	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

[1] "Petrifilm AC" includes two participants that incubated on Petrifilm RAC.

[2] One participant that incubated on Compact Dry TC followed ISO 11737-1:2018 ("Sterilization of health care products").





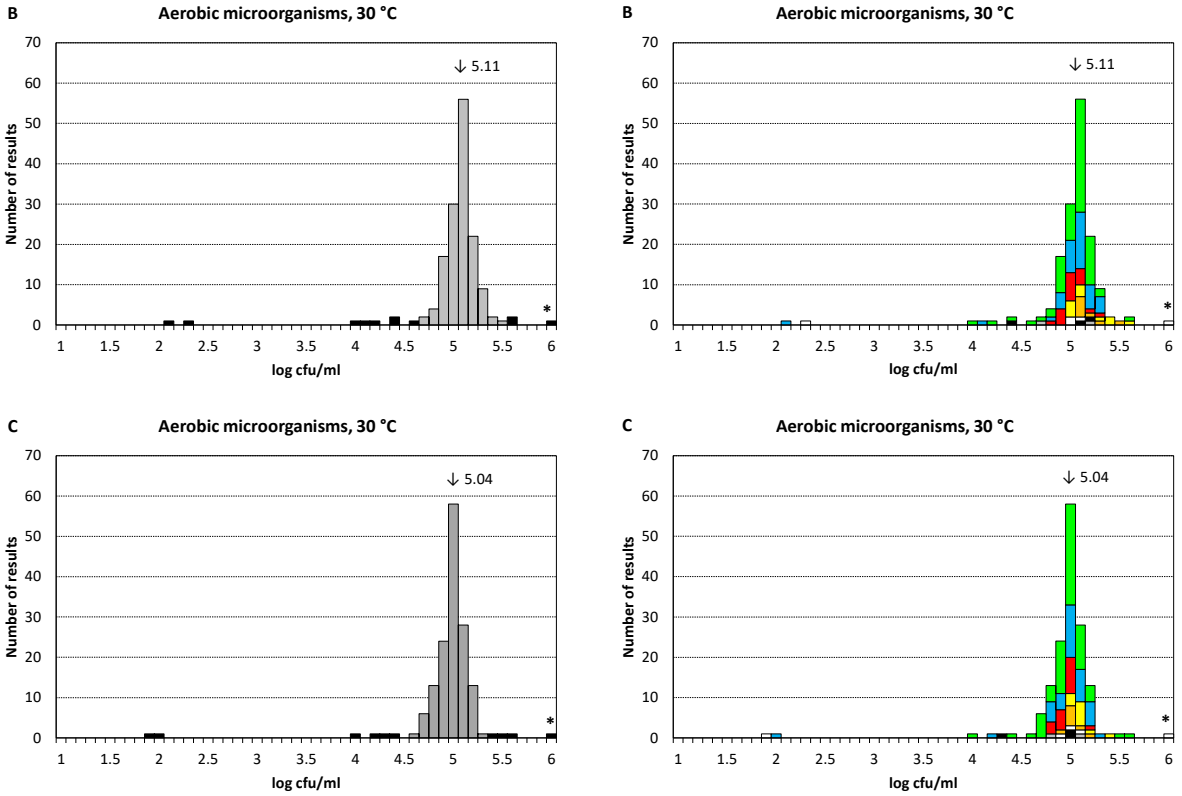


Figure 1. Results from analysis of aerobic microorganisms, 30 °C.

Table 3. Results from analysis of aerobic microorganisms, 20 °C.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**26**	**25**	**5.05**	**0.15**	**0**	**1**	**0**	**27**	**25**	**5.13**	**0.14**	**0**	**2**	**0**	**28**	**27**	**5.08**	**0.12**	**0**	**1**	**0**
PCA	16	15	5.04	0.13	0	1	0	17	16	5.11	0.10	0	1	0	18	17	5.08	0.11	0	1	0
TEMPO AC	5	5	5.08	0.17	0	0	0	5	5	5.23	0.13	0	0	0	5	5	5.12	0.10	0	0	0
Petrifilm AC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
IA	2	2	-	-	0	0	0	2	1	-	-	0	1	0	2	2	-	-	0	0	0
YGC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

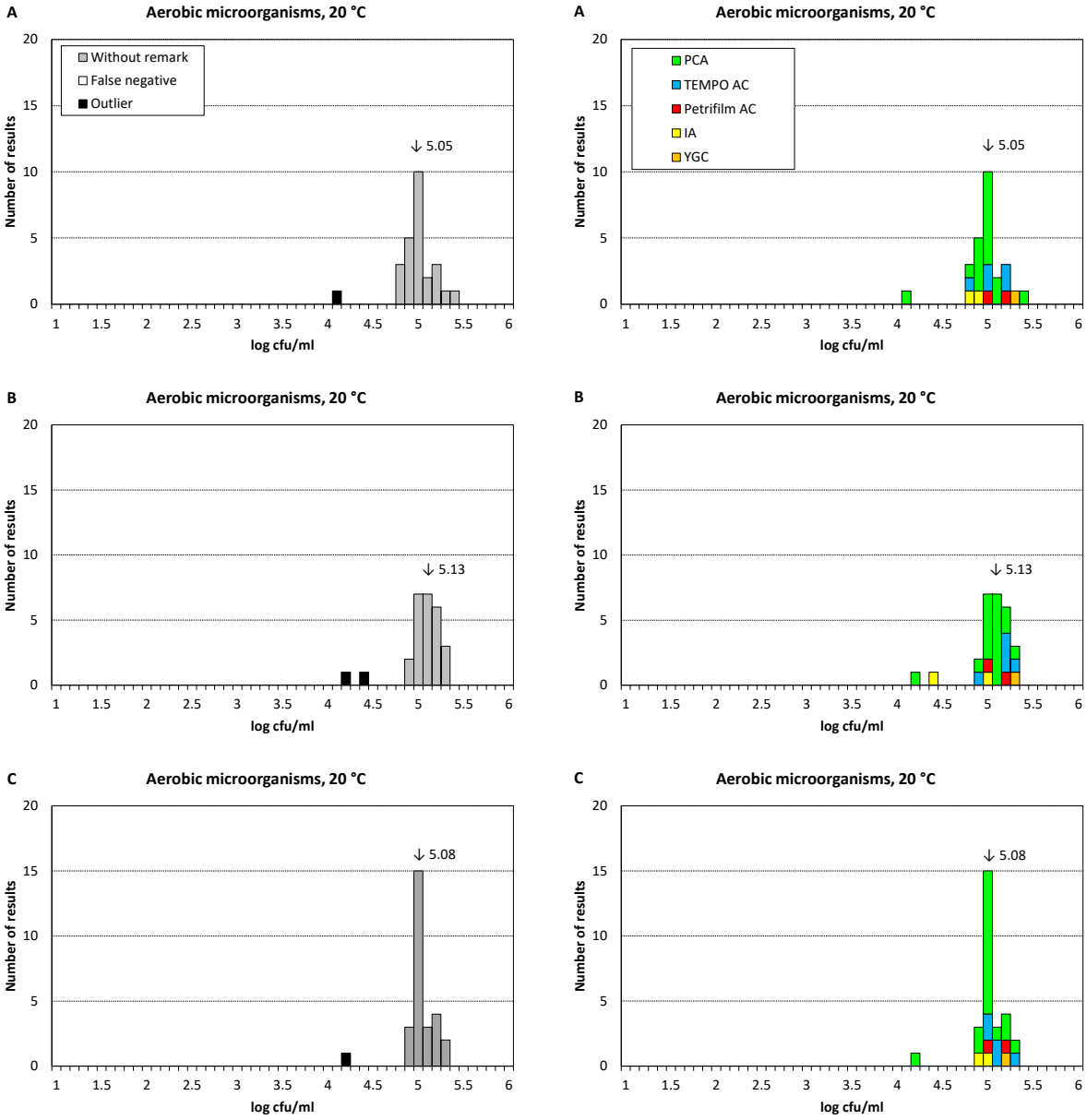


Figure 2. Results from analysis of aerobic microorganisms, 20 °C.

Contaminating microorganisms

Sample A

All strains in the sample were target organisms, and were present in similar concentrations. *B. cereus, E. coli* and *S. aureus* are catalase-positive, whereas *E. durans* is catalase-negative. It may therefore be excluded if a catalase test is performed.

Due to the high measurement uncertainty of the assigned value, all 15 reported results are considered acceptable.

Note: The measurement uncertainty of the assigned value is not negligible. The evaluation of the results could therefore be affected. As a consequence, the lower acceptance limit has been manually adjusted so that one value – initially assessed as a low outlier – is included among the accepted results.

Sample B

All strains in the sample were target organisms. The strain of *S. xylosus* was present in a higher concentration than *B. cereus* and *E. durans*. *B. cereus* and *S. xylosus* are catalase-positive, whereas *E. durans* is catalase-negative. It may therefore be excluded if a catalase test is performed.

Due to the high measurement uncertainty of the assigned value, and a relatively high number of potential low outliers, all 15 reported *positive* results are considered acceptable. One false negative result was reported.

Note: The results had a fairly wide distribution. Therefore, three low results were excluded when determining x_{PT} and s_{PT}. Despite this, the measurement uncertainty of the assigned value (u_{PT}) is not negligible. This, combined with a low number of evaluable results, makes the statistical assessment of the results uncertain. The lower acceptance limit has therefore been manually adjusted, so that all reported positive results are considered acceptable.

Sample C

The sample was identical to sample A.

Due to the high measurement uncertainty of the assigned value, all 15 reported results are considered acceptable.

Note: The measurement uncertainty of the assigned value is not negligible. The evaluation of the results could therefore be affected. As a consequence, the acceptance limit have been manually adjusted so that two values – initially assessed as a low and a high outlier, respectively – are included among the accepted results.

General remarks

Only 16 participants reported results, and the statistical analysis was therefore based on a somewhat limited dataset. As a consequence, and also considering that the measurement uncertainties of the assigned values were not negligible, all positive results are considered acceptable.

Ten of the 16 participants followed ISO 13559:2002 / IDF 153:2002. This was last reviewed by ISO in 2019 and remains current. One participant followed a modified version of the older IDF 153:1999. All participants except three incubated on SFA.

The goal of the analysis is to identify potential contaminating microorganisms in dairy products. For these products, lactic acid bacteria are generally not considered as contaminating microorganisms. Lactic acid bacteria are catalase-negative and some participants therefore use confirmation with a catalase test. Such a test is however not strictly necessary with ISO 13559:2002 / IDF 153:2002.

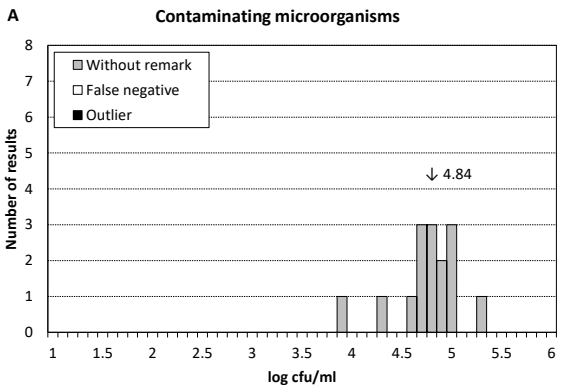
Overall, the results for the identical samples A and C were highly similar.

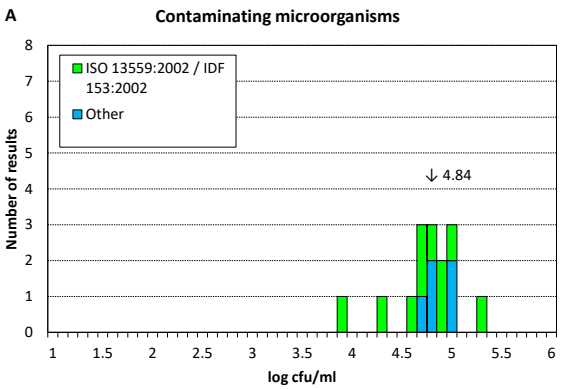
Table 4. Results from analysis of contaminating microorganisms.

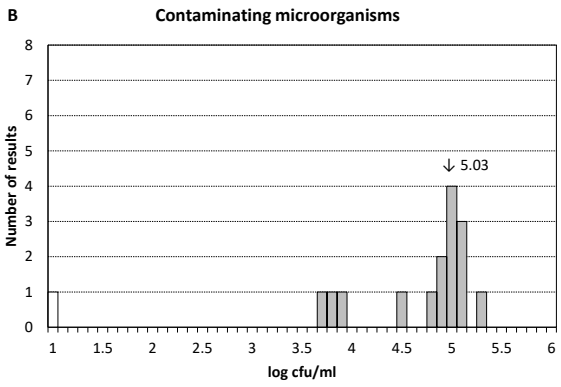
Method	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**15**	**15**	**4.84**	**0.22**	**0**	**0**	**0**	**16**	**15**	**5.03**	**0.15**	**1**	**0**	**0**	**15**	**15**	**4.81**	**0.18**	**0**	**0**	**0**
ISO 13559:2002 / IDF 153:2002[a]	10	10	4.80	0.39	0	0	0	11	10	4.95	0.52	1	0	0	10	10	4.75	0.41	0	0	0
Other	5	5	4.85	0.16	0	0	0	5	5	5.04	0.56	0	0	0	5	5	4.90	0.10	0	0	0

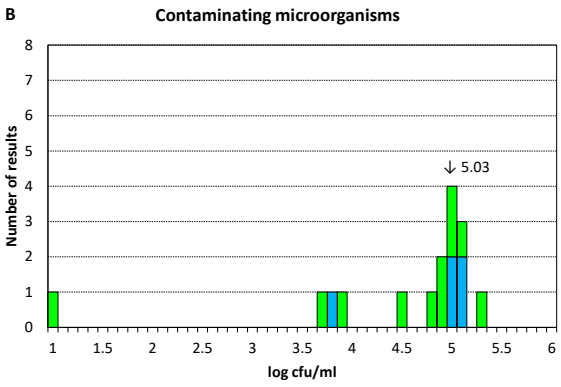
For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

[a] Includes one participant that used a modified version of IDF 153:1991.









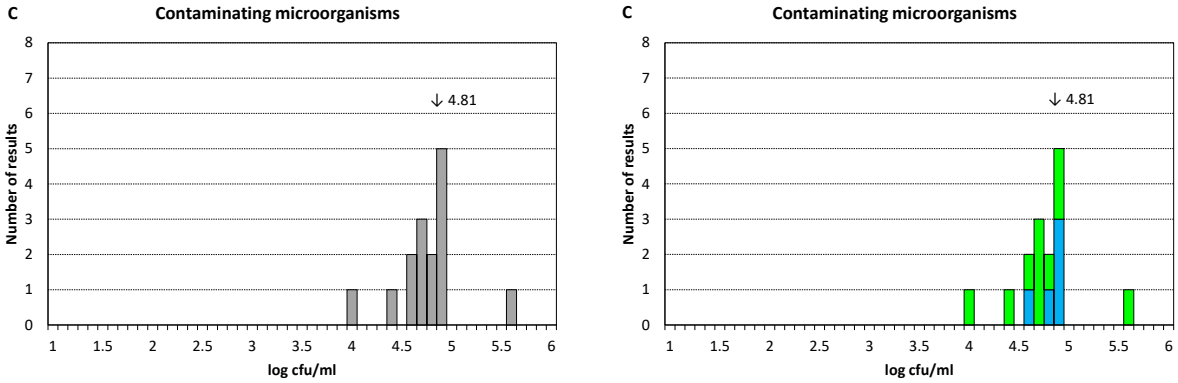


Figure 3. Results from analysis of contaminating microorganisms.

Enterobacteriaceae

Sample A

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

In total, 138 participants reported results. Six low and two high outliers were reported.

Sample B

No target organism was present in the sample.

In total, 139 participants reported results. All results were correct negative.

Sample C

The sample was identical to sample A.

In total, 138 participants reported results. Four low and two high outliers were reported.

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 (41 %), a method with Petrifilm EB (27 %), or an ISO method (19 %). Among the latter, the majority followed ISO 21528-2:2017, which is based on colony-count. In comparison, 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}. Both ISO standards were last reviewed by ISO in 2022 and remain current. Nine participants still followed either of the previous – and now withdrawn – ISO 21528-2:2004 and ISO 21528-1:2004.

Confirmation was performed by 56 % of the participants, and most often consisted of an oxidase test.

Table 5. Results from analysis of Enterobacteriaceae.

Method	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**138**	**130**	**4.37**	**0.20**	**0**	**6**	**2**	**139**	**139**	**-**	**-**	**0**	**-**	**-**	**138**	**132**	**4.37**	**0.20**	**0**	**4**	**2**
VRBG	82	77	4.37	0.22	0	3	2	83	83	-	-	0	-	-	82	78	4.31	0.21	0	3	1
Petrifilm EB	39	38	4.40	0.12	0	1	0	39	39	-	-	0	-	-	39	37	4.48	0.09	0	1	1
TEMPO EB	10	10	4.54	0.09	0	0	0	10	10	-	-	0	-	-	10	10	4.54	0.16	0	0	0
TSA/VRBG	3	1	-	-	0	2	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Other	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
Compact Dry ETB	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

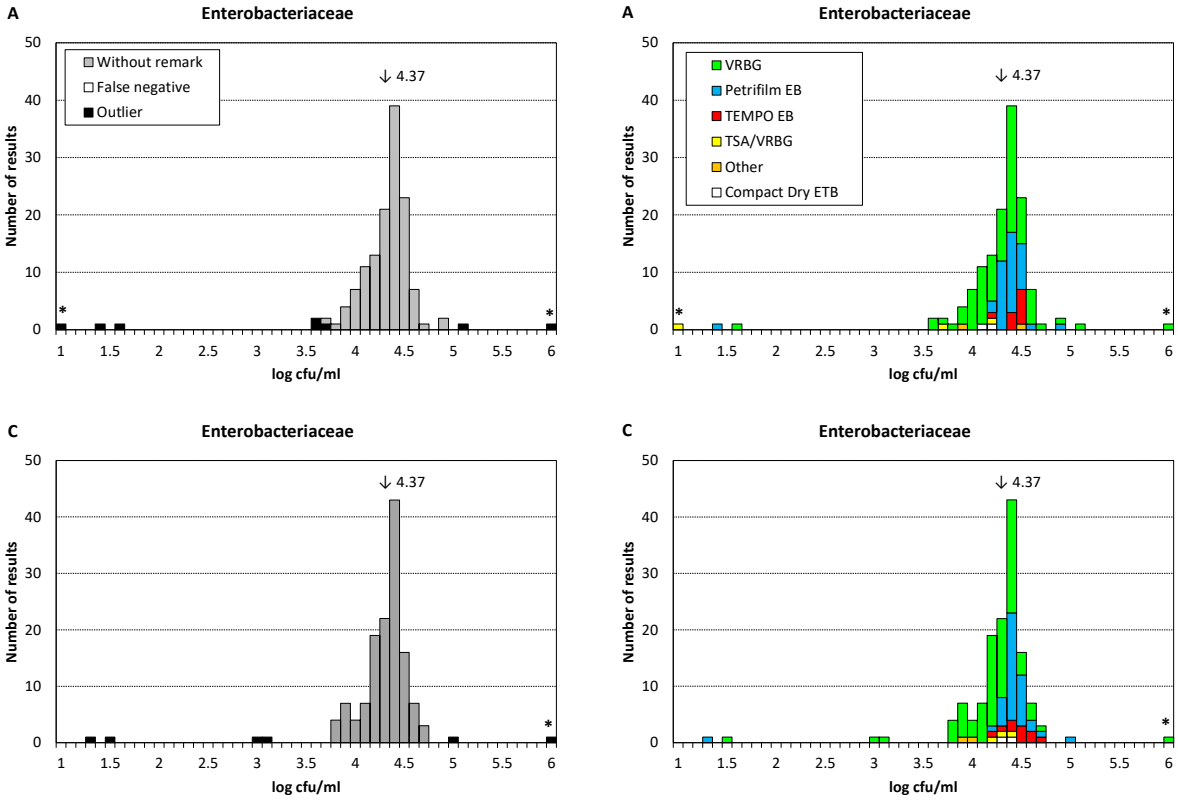


Figure 4. Results from analysis of Enterobacteriaceae

Coliform bacteria, 30 °C and 37 °C

Sample A

E. coli was target organism. On VRB, it forms typical red colonies surrounded by a bile salt precipitation zone. The strain is oxidase-negative and produces gas from lactose fermentation in BGLB.

For the analysis at 30 °C, 38 participants reported results. Two false negative results were reported.

For the analysis at 37 °C, 85 participants reported results. Two low outliers were reported, as well as two false negative results.

Sample B

No target organism was present in the sample.

For the analysis at 30 °C, 40 participants reported results. All results were correct negative.

For the analysis at 37 °C, 86 participants reported results. All results were correct negative.

Sample C

The sample was identical to sample A.

For the analysis at 30 °C, 40 participants reported results. One low outlier was reported, as well as two false negative results.

For the analysis at 37 °C, 86 participants reported results. Three low outliers were reported, as well as one false negative result.

General remarks

Coliform bacteria are Gram-negative rods that ferment lactose with the production of gas and acid by-products. On VRB, they form characteristic red colonies due to uptake of crystal violet and neutral red from the medium. The colonies are normally surrounded by a red/pink precipitation zone, which is formed due to the precipitation of bile salts when the pH decreases. Petrifilm CC and Petrifilm EC/CC are based on VRB, but also have a plastic film that facilitates detection of gas production.

Most participants followed NMKL 44:2004 or ISO 4832:2006, which both use VRB as the primary medium. At 37 °C, 3M™ Petrifilm™ was also used by many participants. Since these methods are based on media with a similar composition, differences in results are most often due to whether presumptive colonies and/or atypical colonies are confirmed or not. This varies between both methods and individual participants that use a particular method. In this PT however, there was no problematic target organism, and the results from the different methods and media were highly similar. ISO 4832:2006 was last reviewed by ISO in 2021, and remains current.

LSB in combination with BGLB was used by participants that followed the MPN-based methods ISO 4831:2006 and NMKL 96:2009. They are adapted for use when the expected concentration of coliform bacteria is low, in the range of 100–300 cfu g^{-1}. This is normally not a problem, even though the

concentrations of coliform bacteria in the PT samples is usually significantly higher. It can however be noted that there were several high results from participants that used LSB/BGLB, for example at 37 °C incubation for sample A. ISO 4831:2006 was last reviewed by ISO in 2021 and remains current.

A few participants used methods/media that detect β-galactosidase and β-glucuronidase activity; RAPID'E.coli 2, Compact Dry EC and Brilliance EC/CC. For example on RAPID'E.coli 2 agar, coliform bacteria (Gal+/Gluc-) form blue/green colonies, while *E. coli* (Gal+/Gluc+) form pink/purple colonies. A few participants performed a pre-incubation on TSA prior to incubation on VRB, which is recommended by some methods if the sample is suspected to contain stressed coliform bacteria.

Overall, the results for the identical samples A and C were highly similar.

Table 6. Results from analysis of coliform bacteria, 30 °C.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**38**	**36**	**4.30**	**0.28**	**2**	**0**	**0**	**40**	**40**	**-**	**-**	**0**	**-**	**-**	**40**	**37**	**4.28**	**0.29**	**2**	**1**	**0**
VRB	28	27	4.22	0.29	1	0	0	29	29	-	-	0	-	-	29	27	4.24	0.27	1	1	0
TSA/VRB	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Petrifilm CC	2	2	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
LSB/BGLB	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
Rapid'E.coli 2	1	0	-	-	1	0	0	1	1	-	-	0	-	-	1	0	-	-	1	0	0
TEMPO CC	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0
Brilliance EC/CC	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

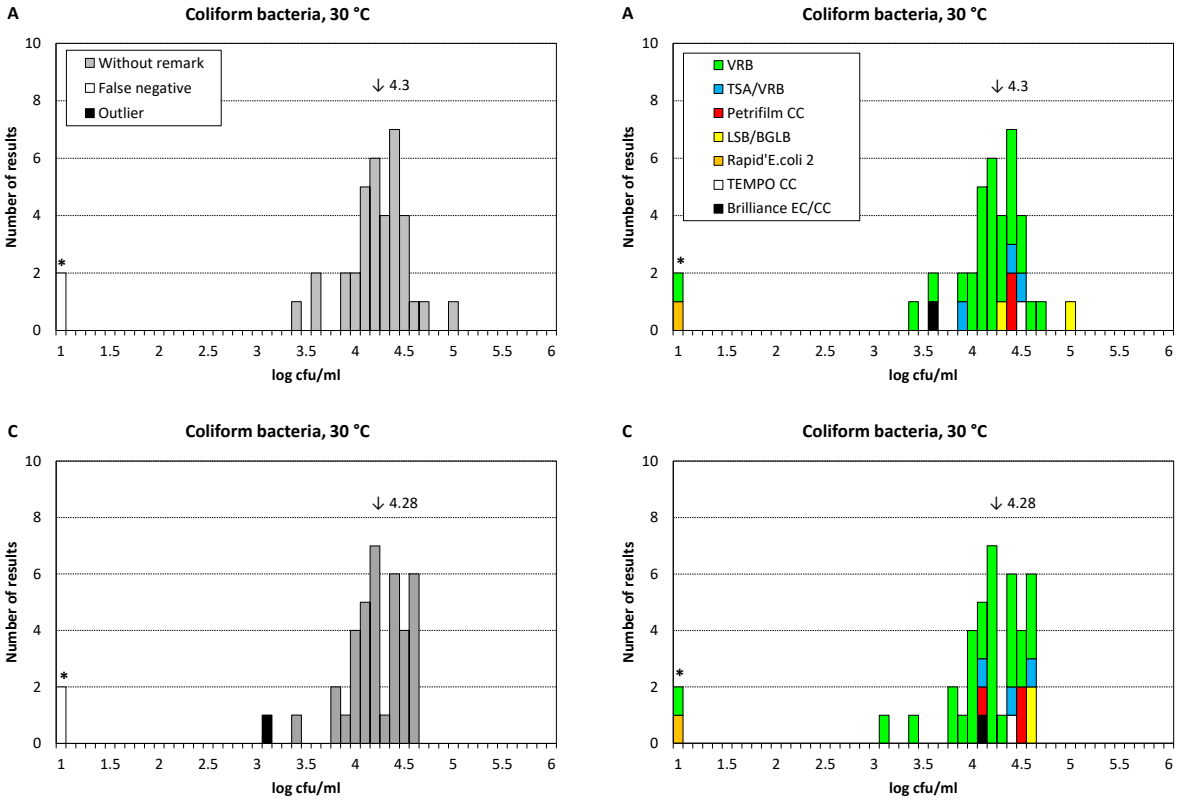


Figure 5. Results from analysis of coliform bacteria, 30 °C.

Table 7. Results from analysis of coliform bacteria, 37 °C.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**85**	**81**	**4.35**	**0.26**	**2**	**2**	**0**	**86**	**86**	**-**	**-**	**0**	**-**	**-**	**86**	**82**	**4.34**	**0.24**	**1**	**3**	**0**
VRB	35	34	4.27	0.31	0	1	0	36	36	-	-	0	-	-	36	34	4.27	0.25	0	2	0
Petrifilm EC/CC	18	17	4.38	0.15	0	1	0	18	18	-	-	0	-	-	18	18	4.45	0.12	0	0	0
Petrifilm CC	10	10	4.50	0.17	0	0	0	10	10	-	-	0	-	-	10	10	4.43	0.12	0	0	0
TSA/VRB	6	6	4.54	0.24	0	0	0	6	6	-	-	0	-	-	6	6	4.43	0.15	0	0	0
LSB/BGLB	5	5	4.96	0.41	0	0	0	5	5	-	-	0	-	-	5	5	4.54	0.50	0	0	0
TEMPO CC	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Rapid'E.coli 2	3	2	-	-	1	0	0	3	3	-	-	0	-	-	3	2	-	-	0	1	0
Other	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Brilliance EC/CC	2	1	-	-	1	0	0	2	2	-	-	0	-	-	2	1	-	-	1	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

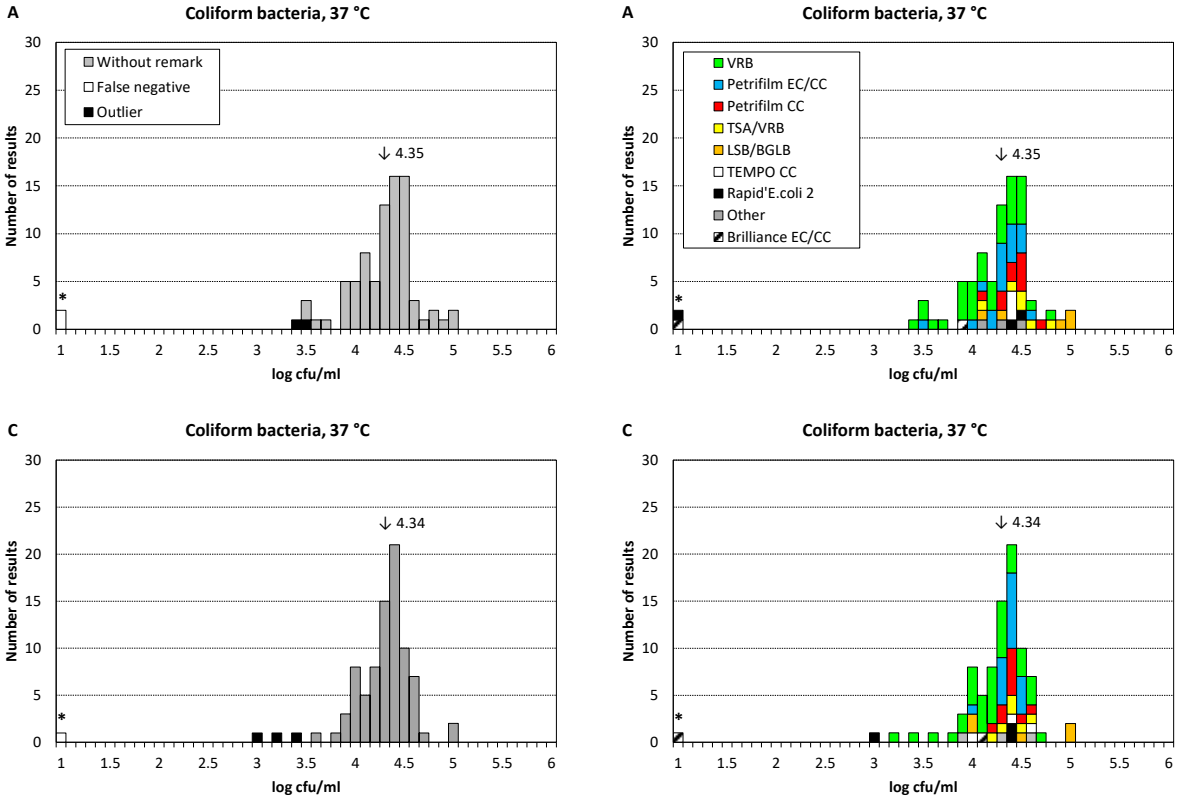


Figure 6. Results from analysis of coliform bacteria, 37 °C.

Thermotolerant coliform bacteria and *Escherichia coli*

Sample A

E. coli was the target organism for both analyses. On VRB, it forms typical red colonies with a bile salt precipitation zone. The strain produces both gas and indole in LTLSB. The strain is oxidase-negative.

For thermotolerant coliform bacteria, 36 participants reported results. No outliers or false negative results were reported.

For *E. coli*, 106 participants reported results. Five low and one high outliers were reported.

Sample B

No target organism was present in the sample.

For thermotolerant coliform bacteria, 38 participants reported results. All results were correct negative.

For *E. coli*, 109 participants reported results. All results were correct negative.

Sample C

The sample was identical to sample A.

For thermotolerant coliform bacteria, 37 participants reported results. No outliers or false negative results were reported.

For *E. coli*, 107 participants reported results. Five low and one high outliers were reported.

General remarks

On VRB, thermotolerant coliform bacteria form dark red colonies, surrounded by a red zone of bile salt precipitation. They also produce gas as a consequence of lactose fermentation. *E. coli* can be distinguished from other thermotolerant coliform bacteria by their production of indole, and since they possess the β-glucuronidase enzyme.

NMKL 125:2005 was the most commonly used method for the analysis of thermotolerant coliform bacteria. It is based on VRB and describes the analysis of both thermotolerant coliform bacteria and of *E. coli*. For *E. coli*, most participants used methods based on 3M™ Petrifilm™ (either Petrifilm EC/CC or Petrifilm SEC), or NMKL 125:2005. Both Petrifilm EC/CC and Petrifilm SEC include substrates that facilitate detection of β-glucuronidase, and therefore *E. coli* form blue-green colonies on these media. The plastic film in Petrifilm EC/CC and Petrifilm SEC also enables detection of gas production due to lactose fermentation.

The ISO 16649-2:2001 method is based on TBX, on which β-glucuronidase-positive *E. coli* form blue colonies. Participants that use TBX often get lower results compared to participants that use other media.

This was clearly seen for the identical samples A and C in this PT round. Lower results for TBX could be a consequence of participants not performing a pre-incubation at a lower temperature. ISO 16649-2:2001 was last reviewed by ISO in 2019 and remains current.

For *E. coli*, the mean value for the MPN-based TEMPO EC is often higher compared to other methods/media. This was however not apparent in this PT round.

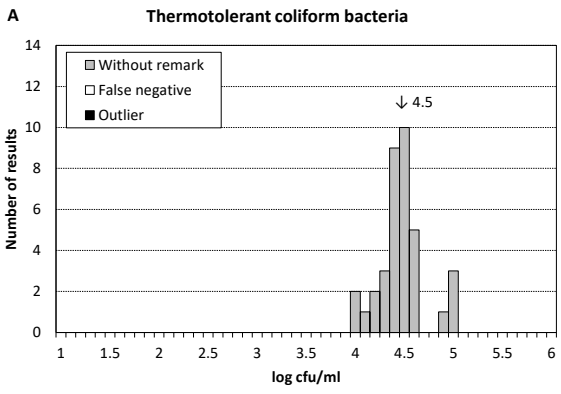
Three participants followed ISO 7251:2005, and typically incubated in LSB/EC. ISO 7251:2005 is an MPN-based method for the detection of *E. coli*. It was last reviewed by ISO in 2019 and remains current. Participants that used LSB/EC reported somewhat higher results compared to other participants for thermotolerant coliform bacteria in sample A. Strangely however, the same was not true for the identical sample C.

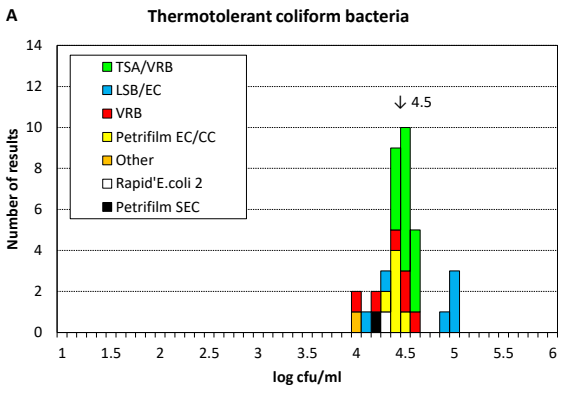
Note: For thermotolerant coliform bacteria, several participants reported using methods that are mainly aimed at detection of *E. coli*. Though this is technically incorrect, since only *E. coli* was present in this PT, and since it cannot be excluded that the participants used modifications and/or confirmation to facilitate enumeration of thermotolerant coliform bacteria, the results were still included in the evaluation.

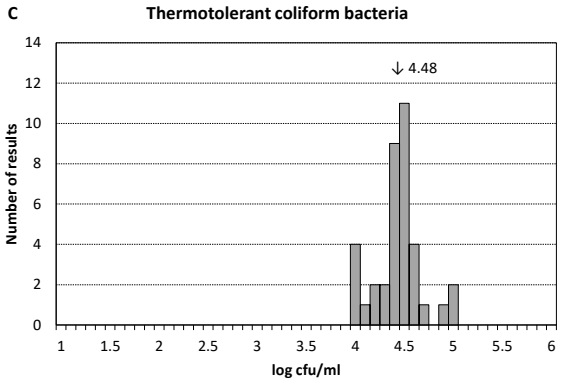
Table 8. Results from analysis of thermotolerant coliform bacteria.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**36**	**36**	**4.50**	**0.19**	**0**	**0**	**0**	**38**	**38**	**-**	**-**	**0**	**-**	**-**	**37**	**37**	**4.48**	**0.19**	**0**	**0**	**0**
TSA/VRB	15	15	4.54	0.08	0	0	0	15	15	-	-	0	-	-	15	15	4.57	0.13	0	0	0
LSB/EC	6	6	4.98	0.38	0	0	0	7	7	-	-	0	-	-	6	6	4.54	0.45	0	0	0
VRB	6	6	4.51	0.22	0	0	0	6	6	-	-	0	-	-	6	6	4.50	0.22	0	0	0
Petrifilm EC/CC	6	6	4.41	0.07	0	0	0	6	6	-	-	0	-	-	6	6	4.49	0.13	0	0	0
Other	1	1	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
Rapid'E.coli 2	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0
Petrifilm SEC	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).







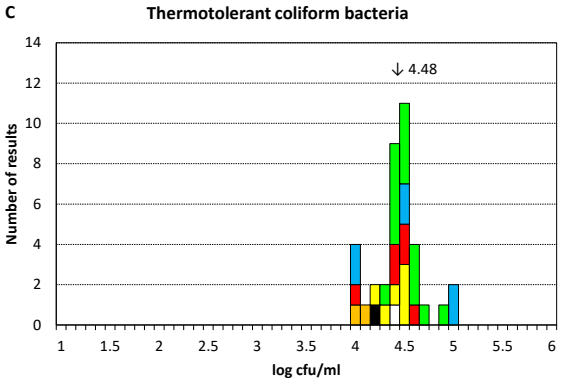


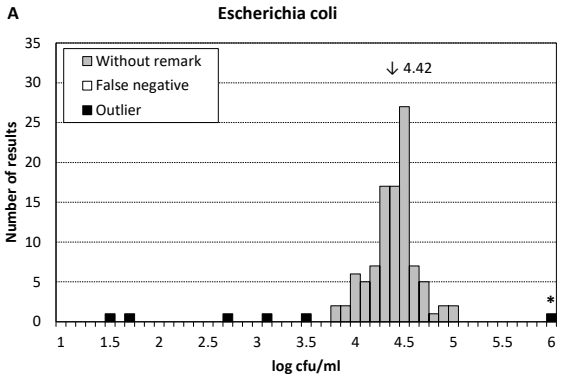
Figure 7. Results from analysis of thermotolerant coliform bacteria.

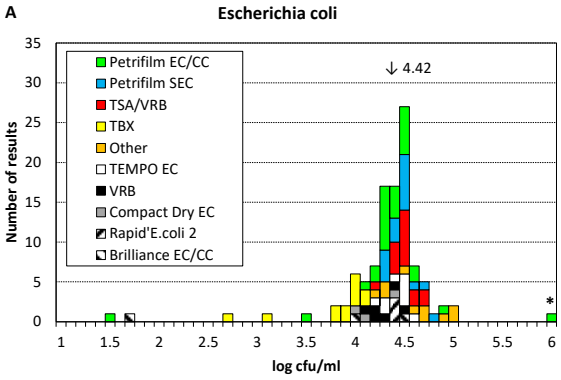
Table 9. Results from analysis of *Escherichia coli*.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**106**	**100**	**4.42**	**0.24**	**0**	**5**	**1**	**109**	**109**	**-**	**-**	**0**	**-**	**-**	**107**	**101**	**4.42**	**0.25**	**0**	**5**	**1**
Petrifilm EC/CC	27	24	4.40	0.16	0	2	1	27	27	-	-	0	-	-	27	26	4.45	0.15	0	1	0
Petrifilm SEC	17	17	4.53	0.15	0	0	0	17	17	-	-	0	-	-	17	16	4.53	0.15	0	0	1
TSA/VRB[1]	16	16	4.54	0.13	0	0	0	16	16	-	-	0	-	-	16	16	4.57	0.16	0	0	0
TBX	12	10	4.02	0.11	0	2	0	13	13	-	-	0	-	-	12	9	3.98	0.11	0	3	0
Other	10	10	4.65	0.30	0	0	0	12	12	-	-	0	-	-	11	11	4.54	0.36	0	0	0
TEMPO EC	9	9	4.52	0.14	0	0	0	9	9	-	-	0	-	-	9	9	4.53	0.06	0	0	0
VRB[1]	6	6	4.31	0.16	0	0	0	6	6	-	-	0	-	-	6	6	4.26	0.17	0	0	0
Compact Dry EC	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Rapid'E.coli 2	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
Brilliance EC/CC	3	2	-	-	0	1	0	3	3	-	-	0	-	-	3	2	-	-	0	1	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

[1] VRB includes participants that used VRBG and TSA/VRB includes participants that used TSA/VRBG.





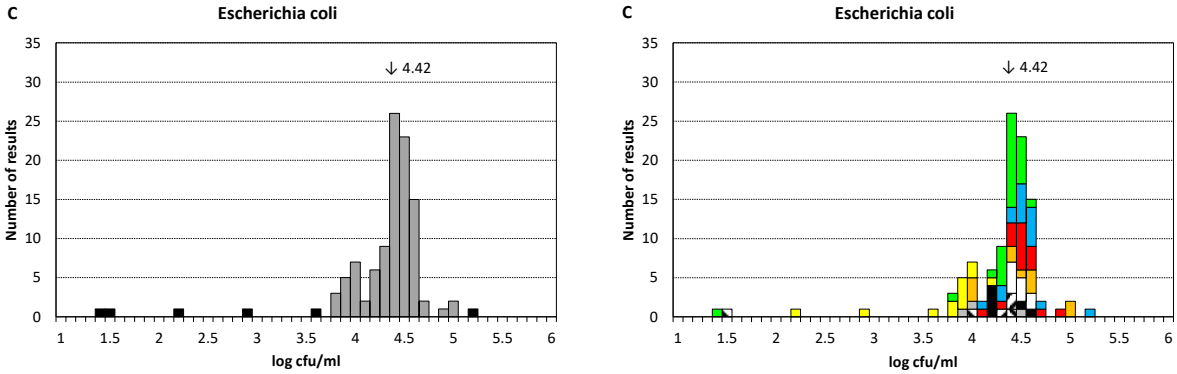


Figure 8. Results from analysis of *Escherichia coli*.

Presumptive *Bacillus cereus*

Sample A

B. cereus was target organism. On BA, it forms typical grey colonies surrounded by a zone of haemolysis. On BcsA, it forms typical blue colonies surrounded by a blue zone of precipitation.

In total, 102 participants reported results. Two low and three high outliers were reported.

Sample B

B. cereus (different strain than in sample A/C) was target organism. On BA, it forms typical grey colonies surrounded by a zone of haemolysis. On BcsA, it forms typical blue colonies surrounded by a blue zone of precipitation.

In total, 102 participants reported results. Three low and two high outliers were reported, as well as five false negative results.

Sample C

The sample was identical to sample A.

In total, 103 participants reported results. Four low and three high outliers were reported.

General remarks

Most participants followed either NMKL 67:2010 (38 %) or ISO 7932:2004 (24 %). The new NMKL 67:2021 – which replaces NMKL 67:2010 – was in contrast only followed only by 14 participants (14 %). Notably, users of the new NMKL 67:2021 reported no false results or outliers, whereas false results and outliers were reported by users of the old NMKL 67:2010 for all three samples. The new version of NMKL 67 stipulates a different primary incubation medium. ISO 7932:2004 was last reviewed by ISO in 2021, and remains current.

The most common selective media were MYP and BcsA. 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. MYP and BcsA were often used together with a non-selective medium, typically BA. On BA, *B. cereus* forms large, irregular grey colonies, surrounded by a distinct zone of haemolysis.

Other selective media that were used included Brilliance™ *B. cereus* agar (CBC), Compact Dry X-BC, BACARA™ and COMPASS® *B. cereus*. Brilliance™ B. cereus 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. Similarly, the chromogenic and selective agents in Compact Dry X-BC cause *B. cereus* to form blue/green colonies, whereas other bacteria normally form white colonies. Compact Dry X-BC may give somewhat lower results compared to the reference method ISO 7932:2004, something that is mentioned in both the NordVal 045 and MicroVal 2011-LR41 validations. PEMBA is

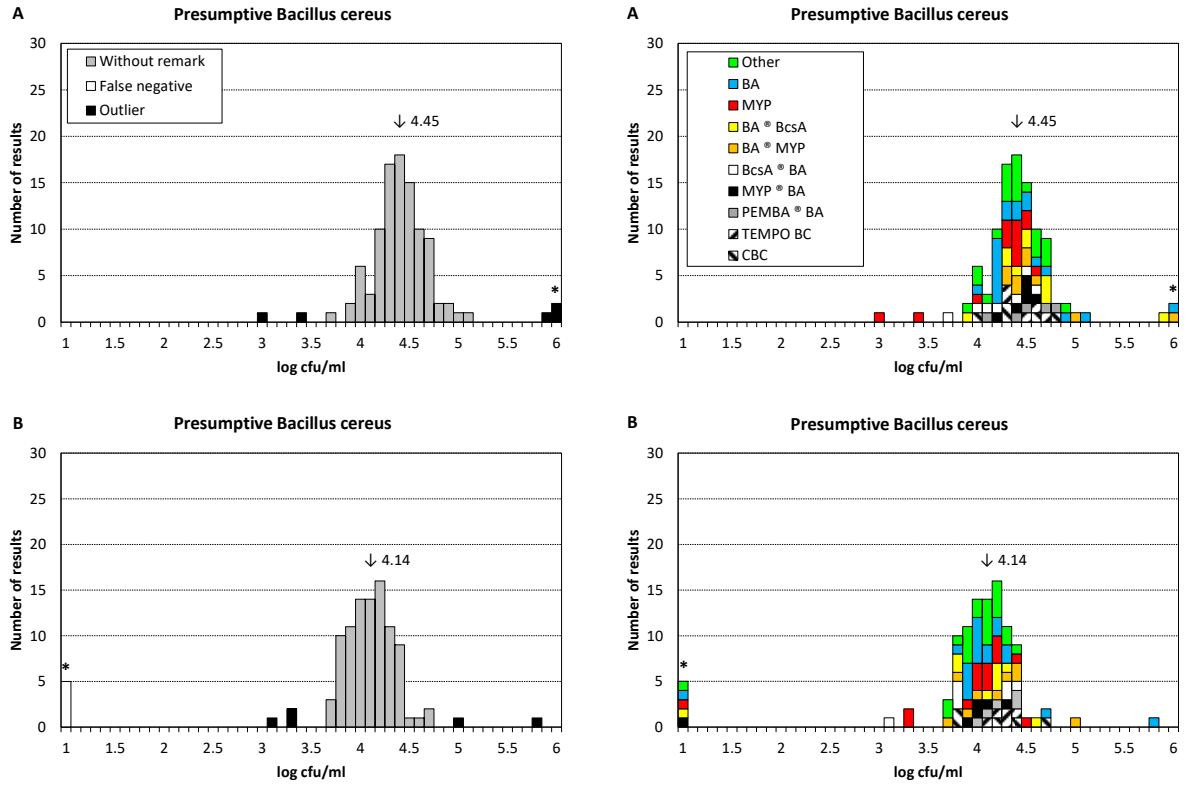
almost identical to BcsA in its composition, and was similarly often used together with BA. In contrast, Brilliance™ B. cereus and Compact Dry X-BC were most often used as standalone media.

Overall, the results for the identical samples A and C were highly similar.

Table 10. Results from analysis of presumptive *Bacillus cereus*.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*N*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**102**	**97**	**4.45**	**0.25**	**0**	**2**	**3**	**102**	**92**	**4.14**	**0.24**	**5**	**3**	**2**	**103**	**96**	**4.45**	**0.26**	**0**	**4**	**3**
Other	22	22	4.45	0.25	0	0	0	22	21	4.11	0.19	1	0	0	22	22	4.43	0.21	0	0	0
BA	19	18	4.37	0.28	0	0	1	19	17	4.05	0.21	1	0	1	19	17	4.41	0.30	0	0	2
MYP	14	12	4.45	0.17	0	2	0	15	12	4.15	0.18	1	2	0	15	12	4.44	0.17	0	3	0
BA → BcsA	10	9	4.57	0.25	0	0	1	9	8	4.24	0.23	1	0	0	10	9	4.51	0.23	0	0	1
BA → MYP	9	8	4.49	0.22	0	0	1	9	8	4.16	0.27	0	0	1	9	8	4.60	0.27	0	1	0
BcsA → BA	7	7	4.23	0.31	0	0	0	7	6	4.08	0.28	0	1	0	7	7	4.49	0.38	0	0	0
MYP → BA	6	6	4.51	0.12	0	0	0	6	5	4.04	0.13	1	0	0	6	6	4.32	0.30	0	0	0
PEMBA ® BA	5	5	4.53	0.25	0	0	0	5	5	4.22	0.18	0	0	0	5	5	4.47	0.14	0	0	0
TEMPO BC	5	5	4.57	0.20	0	0	0	5	5	4.32	0.10	0	0	0	5	5	4.56	0.18	0	0	0
CBC	5	5	4.38	0.30	0	0	0	5	5	4.24	0.38	0	0	0	5	5	4.52	0.15	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).



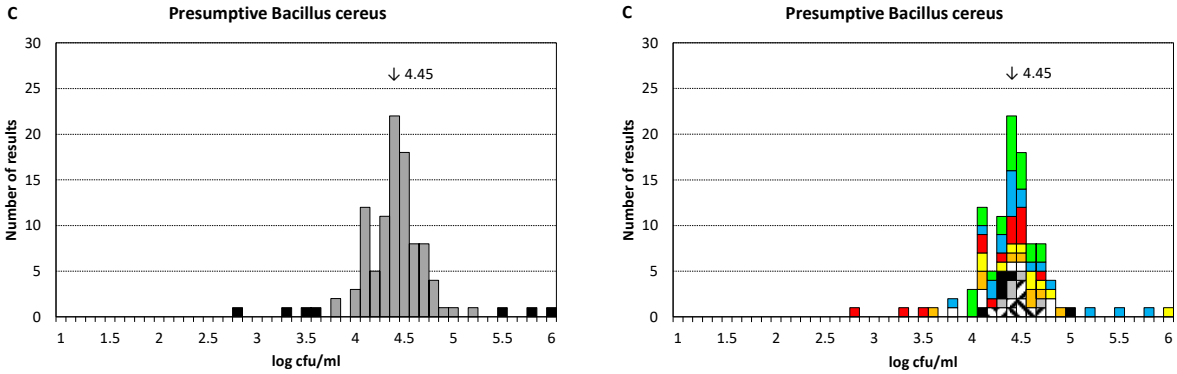


Figure 9. Results from analysis of presumptive *Bacillus cereus*.

Coagulase-positive staphylococci

Sample A

S. aureus was target organism. It forms typical colonies on RPFA. The surrounding coagulase zone may be less prominent after 48 hours incubation, compared to after 24 hours incubation.

In total, 89 participants reported results. Seven low and one high outliers were reported, as well as three false negative results.

Sample B

No target organism was present in the sample. It did however contain *S. xylosus*, which is false positive for the analysis. *S. xylosus* is coagulase-negative and on RPFA, it forms grey colonies without a coagulase zone.

In total, 89 participants reported results. Six false positive results were reported.

Sample C

The sample was identical to sample A.

In total, 90 participants reported results. Eight low outliers were reported, as well as three false negative results.

General remarks

Most participants incubated on BP. On this medium, *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. Participants that used BP typically performed a confirmation based on coagulase activity, for example a tube coagulase test or the use of RPFA as a secondary medium.

The second most common media were RPFA and Petrifilm Staph. With RPFA, the coagulase activity is 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. Participants that used Petrifilm Staph typically performed a confirmation with Petrifilm Staph Express Disk. This 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.

Most participants (40 %) followed NMKL 66:2009, where incubation is done either with BP and/or RPFA. In comparison, ISO 6888-1:2021 stipulates BP, whereas 6888-2:2021 stipulates the use of RPFA. Most participants that followed an ISO method, referenced the withdrawn versions ISO 6888-1:1999 and ISO 6888-2:1999. 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. This was last reviewed by ISO in 2022 and remains current.

Nine participants used a method with TEMPO STA. These participants reported lower results for the identical samples A and C. For sample B, the results were however similar to other methods. This suggests that the strain of *S. aureus* in samples A/C may have been difficult to detect with TEMPO STA.

Overall, the results for the identical samples A and C were highly similar.

Table 11. Results from analysis of coagulase-positive staphylococci.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*N*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**89**	**78**	**4.47**	**0.14**	**3**	**7**	**1**	**89**	**83**	**-**	**-**	**6**	**-**	**-**	**90**	**79**	**4.46**	**0.15**	**3**	**8**	**0**
BP	41	39	4.49	0.13	0	1	1	40	38	-	-	2	-	-	41	39	4.52	0.14	1	1	0
RPFA	18	14	4.52	0.17	3	1	0	19	19	-	-	0	-	-	19	15	4.49	0.11	2	2	0
Petrifilm Staph	15	15	4.49	0.09	0	0	0	15	13	-	-	2	-	-	15	15	4.45	0.11	0	0	0
TEMPO STA	9	4	-	-	0	5	0	9	8	-	-	1	-	-	9	5	4.23	0.18	0	4	0
Compact Dry X-SA	3	3	-	-	0	0	0	3	2	-	-	1	-	-	3	2	-	-	0	1	0
Other	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
EASY Staph®	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

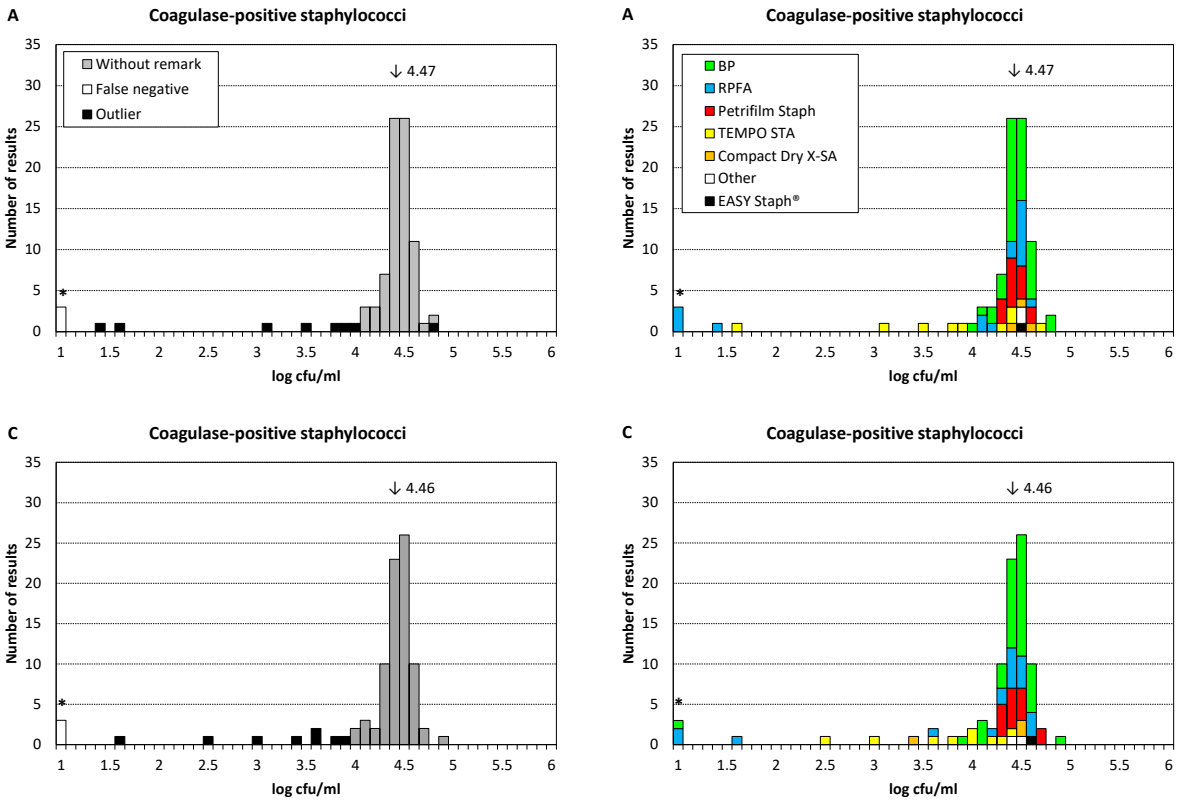


Figure 10. Results from analysis of coagulase-positive staphylococci.

Enterococci

Sample A

E. durans was target organism. On ENT, it forms typical brown-red raised colonies. On BEA, a black colour is typically seen after both 2 h and 24 h incubation.

In total, 54 participants reported results. Two low and one high outliers were reported, as well as three false negative results.

Sample B

E. durans (the same strain as in sample A/C) was target organism. On ENT, it forms typical brown-red raised colonies. On BEA, a distinct black colour is typically seen after both 2 h and 24 h incubation. The strain is catalase-negative.

In total, 55 participants reported results. Four low and one high outliers were reported.

Sample C

The sample was identical to sample A.

In total, 55 participants reported results. Two low and one high outliers were reported, as well as one false negative result.

General remarks

Enterococci are normally defined as Gram-positive, catalase-negative and oval cocci that hydrolyse esculin at 44 °C. On ENT they reduce the colourless substrate 2,3,5-trifenyltetrazolium chloride to red formazan and form slightly raised colonies with a pink/red/maroon colour. They can sometimes also have a colourless edge. On BEA, which is often used for confirmation, enterococci cause a tan/black colour in the medium after 2–24 hours. The colour comes from β-glucosidase hydrolysis of esculin in BEA. This produces esculetin and glucose, which together with iron ions in the medium form a black precipitate.

The vast majority of the participants incubated on ENT, occasionally with a pre-incubation on TSA. Pre-incubation on TSA can be preferable if the presence of stressed enterococci is expected. A smaller number of participants used COMPASS® Enterococcus agar, KEAA or Compact Dry ETC. KEAA was used by participants that followed IDF 149A:1997. With KEAA, hydrolysis of esculin is detected directly in the medium. Similar to BEA, COMPASS also detects β-glucosidase activity, but is instead based on the substrate X-Gluc. On this medium, enterococci therefore form blue colonies. Most participants that incubated on COMPASS also performed a confirmation on BEA.

A clear majority of the participants (65 %) followed NMKL 68:2011. Of the remaining participants, seven followed the drinking water method ISO 7899-2:2000, four IDF 149A:1997 (7 %) and one the withdrawn NMKL 68:2004. ISO 7899-2:2000 was last reviewed by ISO in 2021 and remains current. IDF 149A:1997 has been replaced by ISO 27205:2010/IDF 149:2010. This was last reviewed by ISO in 2020 and remains current.

Overall, the results for the identical samples A and C were highly similar.

Table 12. Results from analysis of enterococci.

Medium	Sample A							Sample B							Sample C						
	N	*n*	m_{PT}	s_{PT}	*F*	<	>	*N*	*N*	m_{PT}	s_{PT}	*F*	<	>	*N*	*n*	m_{PT}	s_{PT}	*F*	<	>
All results	**54**	**48**	**4.46**	**0.15**	**3**	**2**	**1**	**55**	**50**	**4.46**	**0.22**	**0**	**4**	**1**	**55**	**51**	**4.45**	**0.22**	**1**	**2**	**1**
ENT	38	34	4.47	0.14	3	0	1	39	38	4.49	0.23	0	0	1	39	37	4.47	0.19	1	0	1
TSA/ENT	5	4	-	-	0	1	0	5	4	-	-	0	1	0	5	4	-	-	0	1	0
COMPASS	4	4	-	-	0	0	0	4	2	-	-	0	2	0	4	4	-	-	0	0	0
Other	3	2	-	-	0	1	0	3	2	-	-	0	1	0	3	2	-	-	0	1	0
KEAA	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
Compact Dry ETC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

For individual methods: m_{PT} = median value and s_{PT} = standard deviation for the particular method (outliers and false results excluded).

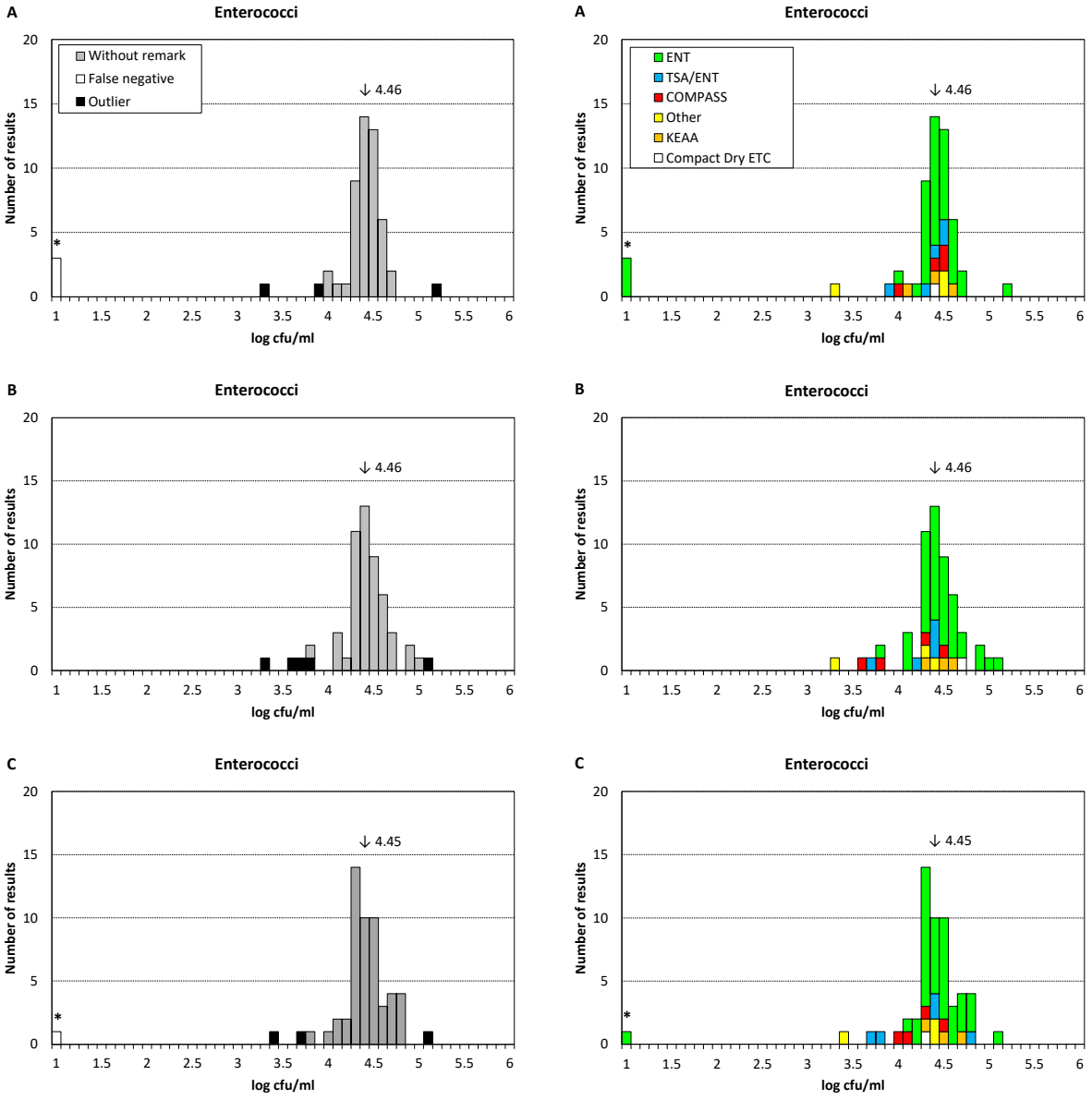


Figure 11. Results from analysis of enterococci.

Gram-negative bacteria in pasteurised milk and cream

Sample A

E. coli is Gram-negative.

In total, 11 participants reported results. All reported a correct positive result.

Sample B

No target organism was present in the sample.

In total, 11 participants reported results. All reported a correct negative result.

Sample C

The sample was identical to sample A.

In total, 11 participants reported results. All reported a correct positive result.

General remarks

NMKL 192:2011 is a qualitative method for detecting recontamination by Gram-negative bacteria in pasteurised milk and cream. These bacteria do not survive high temperature/short time pasteurisation (HTST), where the temperature is raised to 72 °C for at least 15 seconds. Presence of Gram-negative bacteria therefore indicates recontamination, something that may limit the shelf-life of the product.

Seven of the eleven participants followed NMKL 192:2011. Two participants followed a company-specific method and one followed ISO 21528-1.

Ten of the eleven participants incubated on VRBG, while one used MacConkey agar.

Table 13. Results from analysis of Gram-negative bacteria in pasteurised milk and cream.

Medium	Sample A			Sample B			Sample C		
	N	n	F	N	n	F	N	n	F
All results	**11**	**11**	**0**	**11**	**11**	**0**	**11**	**11**	**0**
VRBG	10	10	0	10	10	0	10	10	0
MacConkey	1	1	0	1	1	0	1	1	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. Outliers and false results are highlighted in yellow and red, respectively, 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 [2].

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.

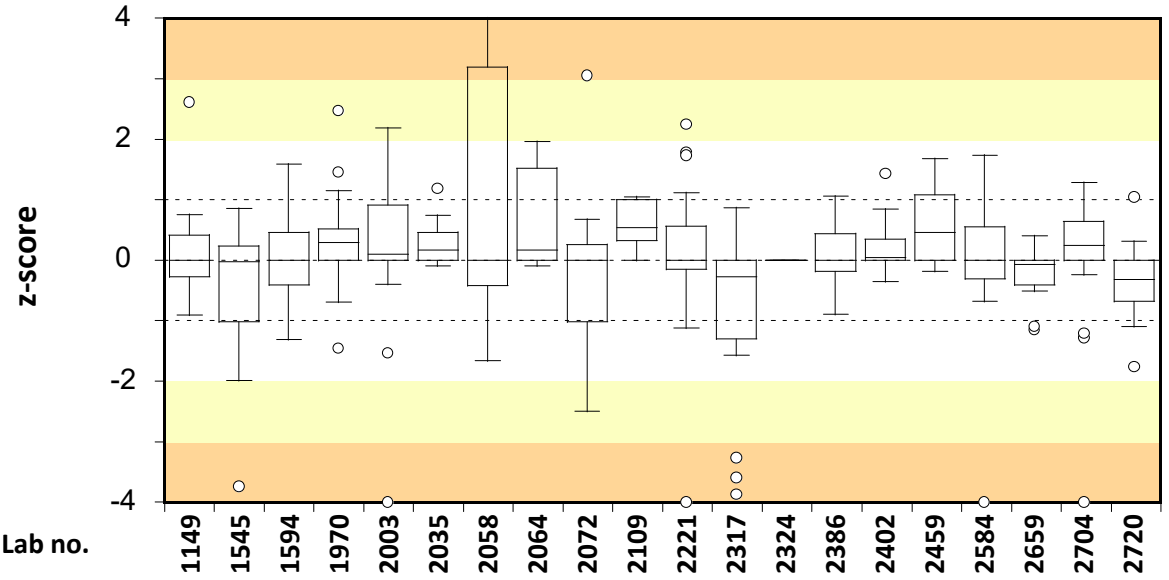
Outliers are included in the figures after being calculated to *z*-scores in the same way as for other results. Correct results for qualitative analyses and correct negative results for quantitative analyses without target organism are given a *z*-score of 0. False results do not generate any *z*-scores, 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.

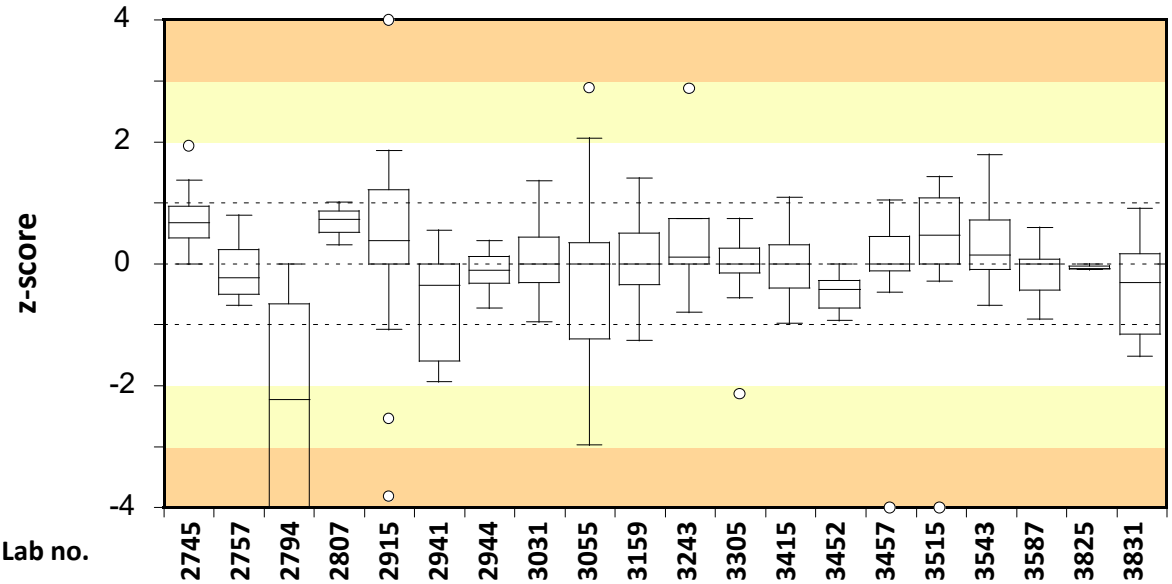
Z-scores >+4 and <−4 are positioned at +4 and −4, respectively, in the plot. The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

* < [lowest value in the box −1.5 × (highest value in the box − lowest value in the box)] or

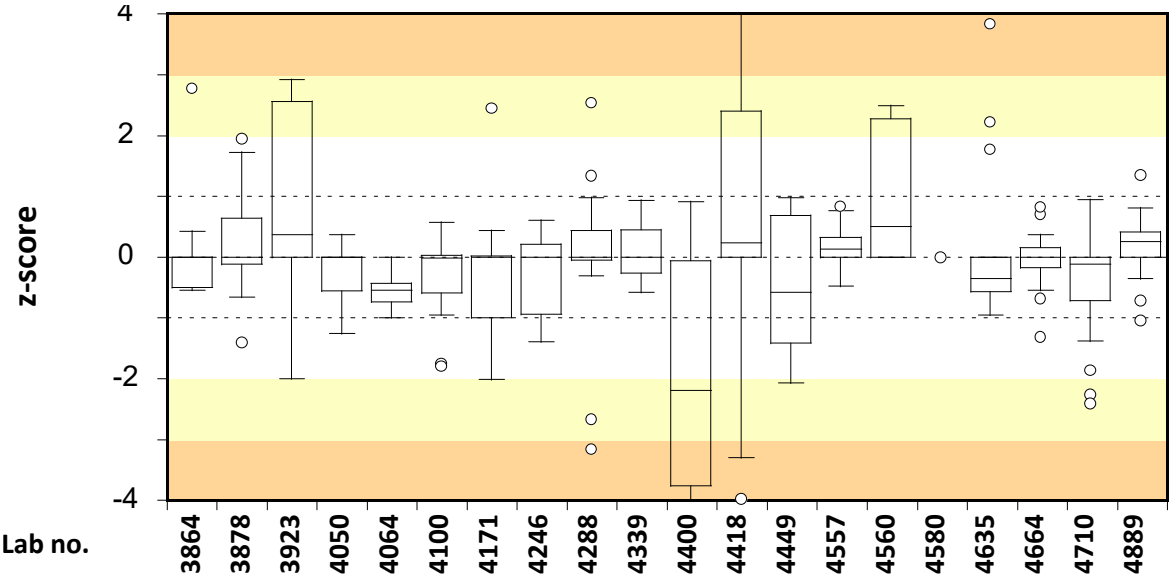
> [highest value in the box + 1.5 × (highest value in the box − lowest value in the box)].



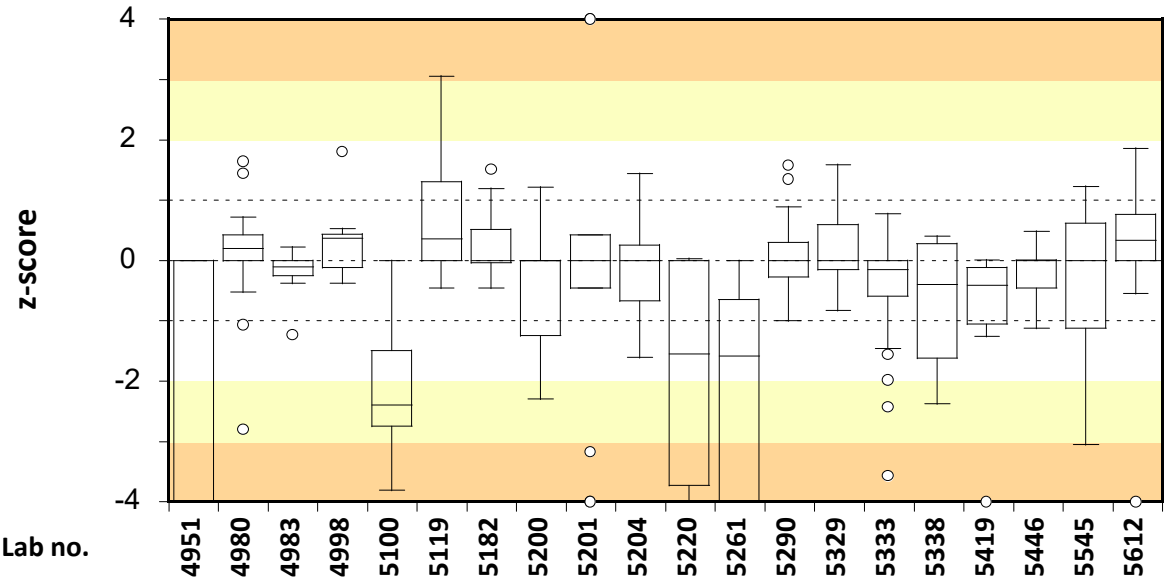
	1149	1545	1594	1970	2003	2035	2058	2064	2072	2109	2221	2317	2324	2386	2402	2459	2584	2659	2704	2720
No. of results	15	21	27	30	24	9	12	9	30	6	27	24	0	18	12	21	20	12	21	9
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Low outliers	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	2	0	1	0
High outliers	0	0	0	0	0	0	4	0	1	0	0	0	0	0	0	0	0	0	0	0



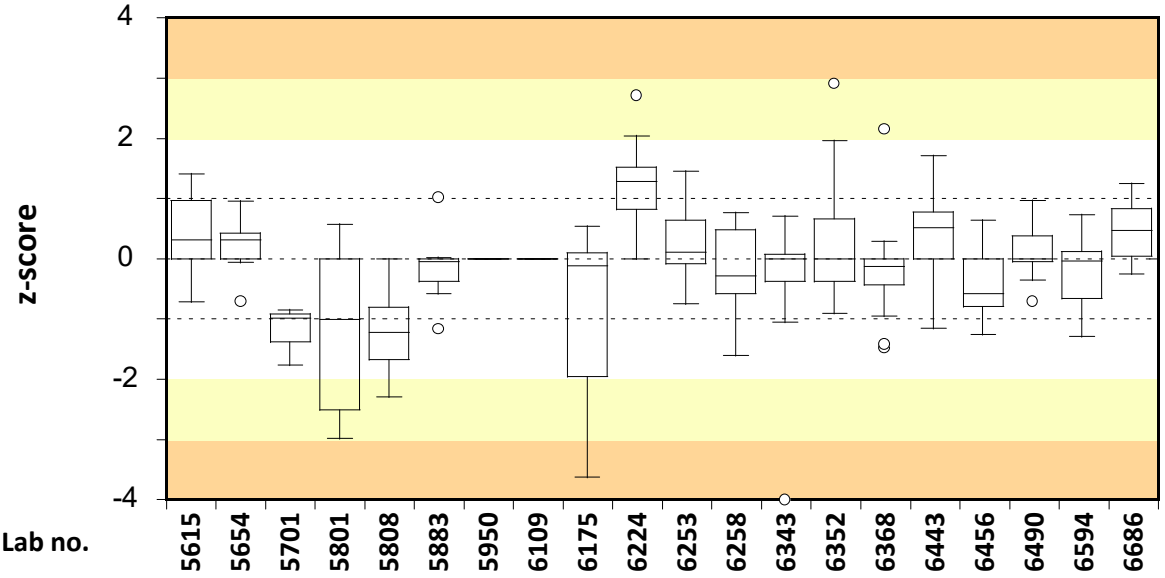
	2745	2757	2794	2807	2915	2941	2944	3031	3055	3159	3243	3305	3415	3452	3457	3515	3543	3587	3825	3831
No. of results	15	12	6	3	18	18	15	12	12	24	6	18	27	6	15	15	18	18	3	12
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Low outliers	0	0	3	0	1	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0
High outliers	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



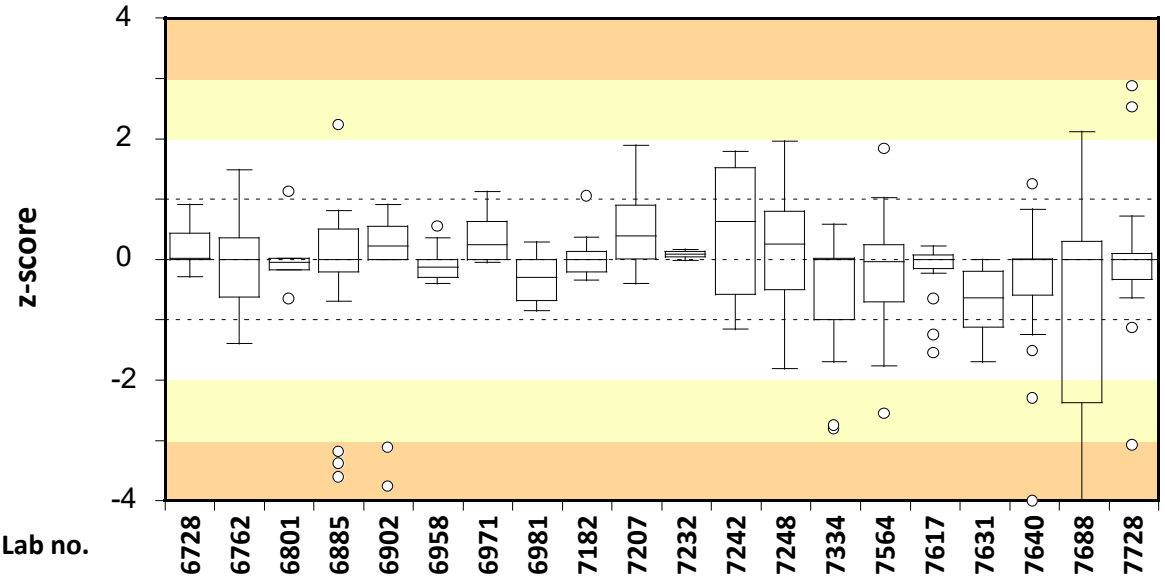
No. of results	9	21	24	15	6	21	17	12	21	24	12	33	9	15	9	9	15	18	18	27
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
False negative	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	0	1	0	4	2	0	0	0	7	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	0	0	0



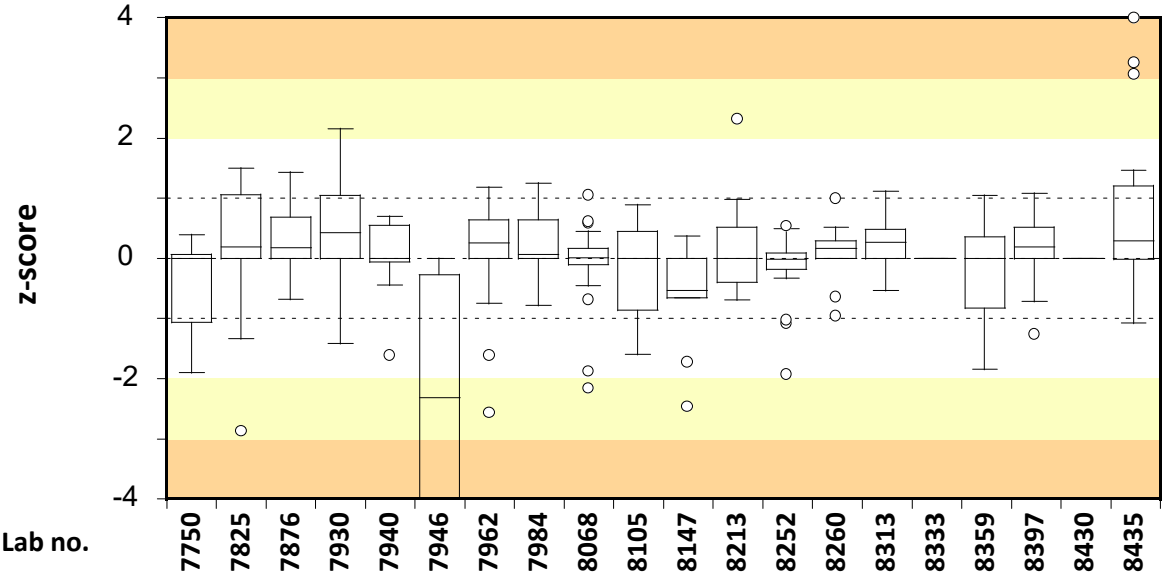
No. of results	12	24	9	9	6	12	15	9	15	30	21	15	21	18	26	6	12	15	18	21
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	2	0	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0
Low outliers	7	0	0	0	1	0	0	0	3	0	6	5	0	0	0	0	2	0	1	2
High outliers	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0



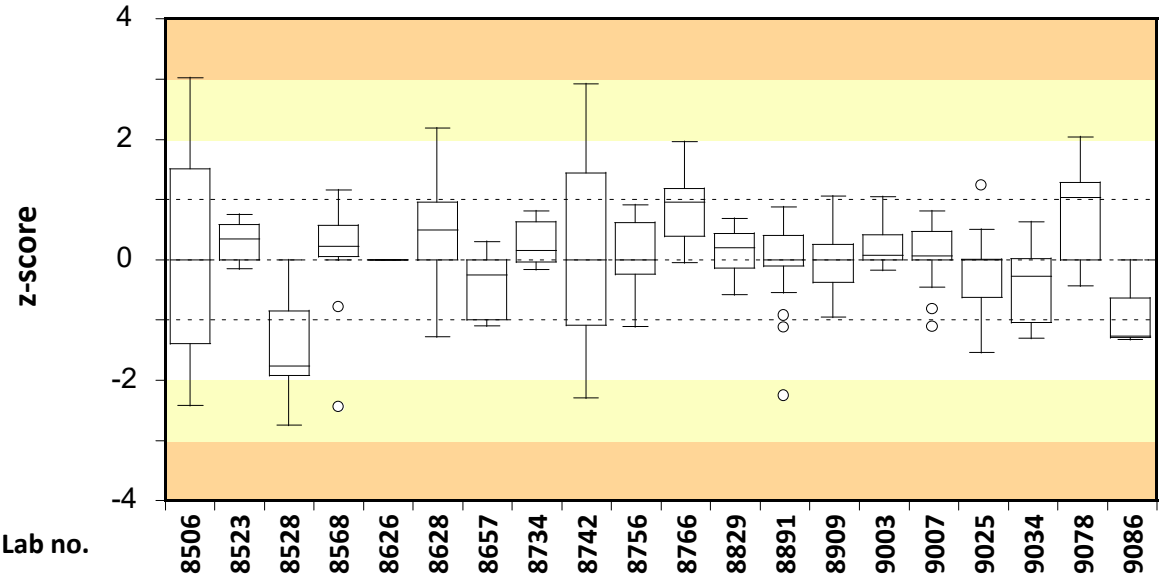
No. of results	18	9	3	9	12	15	0	0	6	9	12	6	18	21	24	9	15	18	9	21
False positive	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
False negative	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



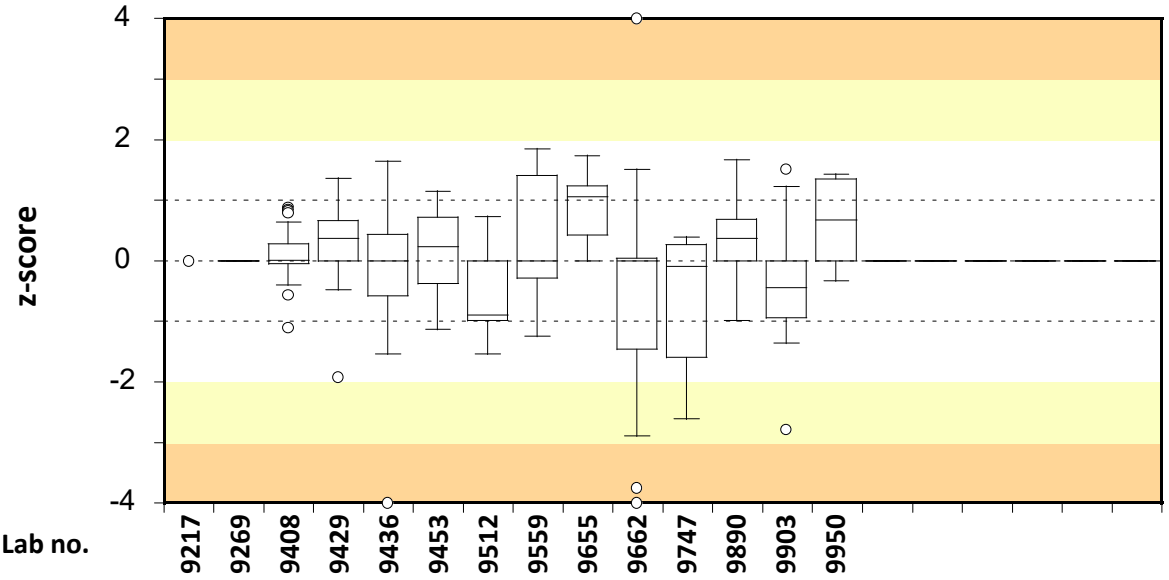
No. of results	15	9	6	21	12	9	6	6	12	12	3	4	33	13	15	15	9	22	27	24
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



	7750	7825	7876	7930	7940	7946	7962	7984	8068	8105	8147	8213	8252	8260	8313	8333	8359	8397	8430	8435
No. of results	12	18	18	21	9	36	27	12	22	15	9	12	15	15	15	0	18	18	0	30
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
False negative	0	2	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3



	8506	8523	8528	8568	8626	8628	8657	8734	8742	8756	8766	8829	8891	8909	9003	9007	9025	9034	9078	9086
No. of results	15	12	9	15	0	30	6	6	15	18	18	6	21	18	15	15	18	12	6	3
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



No. of results	9	0	24	18	27	18	9	24	18	30	9	18	18	6	0	0	0	0	0	0
False positive	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
High outliers	8	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0

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 [3]. 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 and approximate concentrations in the samples. Samples A and C are identical.

Sample	Microorganism	Strain			
		SLV no.[1]	Origin	Reference[2]	$\log_{10}$ cfu ml^{-1}
A	*Bacillus cereus*	SLV-202	Chicken	CCUG 45144	4.5
	Escherichia coli	SLV-085	Water	-	4.5
	Enterococcus durans	SLV-078	Fresh meat	CCUG 44816	4.4
	Staphylococcus aureus	SLV-185	Chicken	CCUG 48090	4.5
B	*Bacillus cereus*	SLV-518	Couscous	CCUG 44741	4.2
	Enterococcus durans	SLV-078	Fresh meat	CCUG 44816	4.5
	Staphylococcus xylosus	SLV-283	Cheese	-	5.0
C	*Bacillus cereus*	SLV-202	Chicken	CCUG 45144	4.5
	Escherichia coli	SLV-085	Water	-	4.5
	Enterococcus durans	SLV-078	Fresh meat	CCUG 44816	4.4
	Staphylococcus aureus	SLV-185	Chicken	CCUG 48090	4.5

[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; Fohm: 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 [4] and [5] respectively.)

Table 15. Concentration mean (m), I_2 and T values from the quality control of the samples; 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	5.05	1.63	2.00	5.17	1.46	1.32	5.05	1.63	2.00
Aerobic microorganisms, 20 °C NMKL method no. 86:2013	4.93	0.26	1.39	5.17	0.78	1.22	4.93	0.26	1.39
Contaminating microorganisms ISO method no. 13559:2002 IDF method no. 153:2002	4.89	1.19	2.06	5.23	**3.18**	1.45	4.89	1.19	2.06
Enterobacteriaceae NMKL method no. 144:2005	4.28	1.62	1.76	-	-	-	4.28	1.62	1.76
Coliform bacteria, 30 ˚C NMKL method no. 44:2004	4.25	1.33	1.72	-	-	-	4.25	1.33	1.72
Coliform bacteria, 37 ˚C NMKL method no. 44:2004	4.26	1.89	1.88	-	-	-	4.26	1.89	1.88
Thermotolerant coliform bacteria NMKL method no. 125:2005	4.55	1.37	1.73	-	-	-	4.55	1.37	1.73
Escherichia coli NMKL method no. 125:2005	4.55	1.37	1.73	-	-	-	4.55	1.37	1.73
Presumptive *Bacillus cereus* NMKL method no. 67:2021	4.50	1.95	1.91	4.18	1.36	1.31	4.50	1.95	1.91
Coagulase-positive staphylococci NMKL method no. 66:2009	4.53	0.73	1.49	5.06	1.18	1.33	4.53	0.73	1.49
Enterococci NMKL method no. 68:2011	4.44	1.36	1.52	4.51	1.00	1.65	4.44	1.36	1.52
Gram-negative bacteria in milk and cream NMKL method no. 192:2011	Pos	-	-	Neg	-	-	Pos	-	-

– No target organism or no value

[1] n = 10 vials analysed in duplicate

[2] n = 5 vials analysed in duplicate

References

1. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Ilbäck J and Blom L. 2023. Protocol – Microbiological Proficiency Testing, Swedish Food Agency.
3. Peterz M and Steneryd AC. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
4. Heisterkamp SH, Hoekstra JA, van Strijp-Lockefeer NGWM, Havelaar AH, Mooijman KA, in't Veld PH, Notermans SHW, Maier EA and Griepink B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
5. Mooijman KM, During M and Nagelkerke NJD 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.

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 participants 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 participants' analytical competence.
- Improved knowledge of analytical methods with respect to various types of organisms.
- Expert support.
- Tool for inspections regarding accreditation.

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

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