

Proficiency testing Food Microbiology

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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
EMB	Eosin Methylene Blue agar
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
MLCB	Manitol Lysine Crystal violet Brilliant green agar
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™ <i>E. coli</i> /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 <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
SCD	Soyabean Casein Digest 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
VIDAS CAM	VIDAS® Campylobacter
VIDAS ECPT	VIDAS® UP E. coli O157 (including H7)
VIDAS LMX	VIDAS® Listeria monocytogens Xpress
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

Enterobacteriaceae

Coliform bacteria, 30 °C

Coliform bacteria, 37 °C

Thermotolerant coliform bacteria

Escherichia coli

Presumptive *Bacillus cereus*

Coagulase-positive staphylococci

Enterococci

Qualitative analyses

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 excluded from the evaluation, or occasionally 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:

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

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 Appendix 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 151 participants: 40 in Sweden, 98 in Europe, and 13 outside of Europe. Individual results are listed in Appendix 1. Z-scores for individual results are listed in Appendix 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			
Microorganisms	<i>Bacillus cereus</i> <i>Escherichia coli</i> <i>Enterococcus durans</i> <i>Staphylococcus aureus</i>				<i>Escherichia coli</i> <i>Proteus mirabilis</i> <i>Staphylococcus aureus</i>				<i>Bacillus cereus</i> <i>Enterococcus faecalis</i> <i>Klebsiella pneumoniae</i> <i>Staphylococcus saprophyticus</i>			
Analysis	Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Aerobic micro-organisms 30 °C	All	152	0	8	All	151	0	8	All	151	0	10
Aerobic micro-organisms 20 °C	All	22	0	1	All	22	0	1	All	21	0	2
Contaminating microorganisms	All	15	0	0	All	14	0	0	All	15	0	0
Enterobacteriaceae	<i>E. coli</i>	132	2	5	<i>E. coli</i> <i>P. mirabilis</i>	132	0	1	<i>K. pneumoniae</i>	131	4	1
Coliform bacteria 30 °C	<i>E. coli</i>	35	0	0	<i>E. coli</i> (<i>P. mirabilis</i>)	36	0	0	<i>K. pneumoniae</i>	38	0	0
Coliform bacteria 37 °C	<i>E. coli</i>	72	3	0	<i>E. coli</i> (<i>P. mirabilis</i>)	72	4	1	<i>K. pneumoniae</i>	69	2	1
Thermotolerant coliform bacteria	<i>E. coli</i>	24	0	0	<i>E. coli</i>	25	0	0	<i>K. pneumoniae</i>	23	2	1
Escherichia coli	<i>E. coli</i>	103	0	3	<i>E. coli</i>	104	2	3	(<i>K. pneumoniae</i>)	105	2	0
Presumptive <i>Bacillus cereus</i>	<i>B. cereus</i>	87	0	1	-	84	2	0	<i>B. cereus</i>	87	0	1
Coagulase-positive staphylococci	<i>S. aureus</i>	83	3	4	<i>S. aureus</i>	83	1	6	(<i>S. saprophyticus</i>)	84	2	0
Enterococci	<i>E. durans</i>	50	0	4	-	49	4	0	<i>E. faecalis</i>	51	1	2
Gram-negative bacteria in milk products	<i>E. coli</i>	8	0	0	<i>E. coli</i> <i>P. mirabilis</i>	8	0	0	<i>K. pneumoniae</i>	9	1	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 are capable of forming colonies on PCA. They were present in similar concentrations.

For the analysis at 30 °C, 152 results were evaluated. Six low and two high outliers were identified.

Note: Six reported results were excluded from the evaluation, since they were not reported correctly.

For the analysis at 20 °C, 22 results were evaluated. One high outlier was identified.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

Sample B

All three strains in the sample are capable of forming colonies on PCA.

For the analysis at 30 °C, 151 results were evaluated. Five low and three high outliers were identified.

Note: Six reported results were excluded from the evaluation, since they were not reported correctly.

For the analysis at 20 °C, 22 results were evaluated. One high outlier was identified.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

Sample C

All strains in the sample are capable of forming colonies on PCA. They were present in similar concentrations.

For the analysis at 30 °C, 151 results were evaluated. Six low and four high outliers were identified.

Note: Six reported results were excluded from the evaluation, since they were not reported correctly.

For the analysis at 20 °C, 21 results were evaluated. One low and one high outliers were identified.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

General remarks

Most participants followed either ISO 4833-1:2013, NMKL 86:2013 or used 3M Petrifilm AC. 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.

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

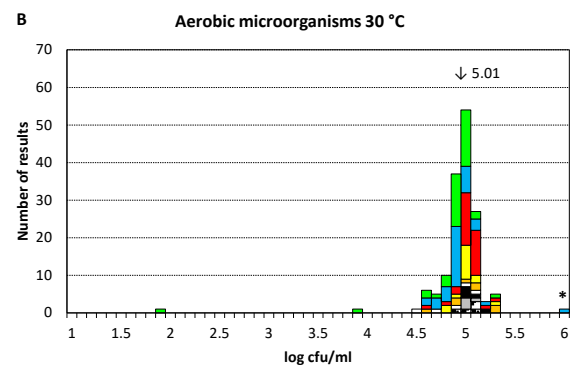
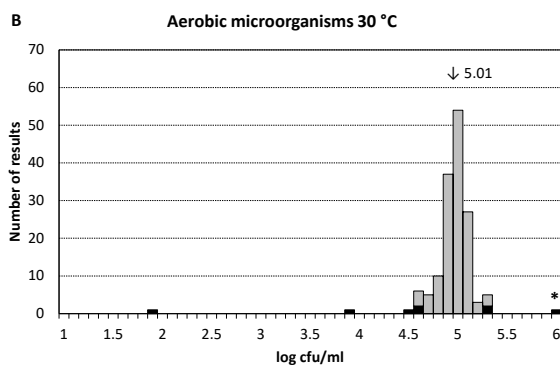
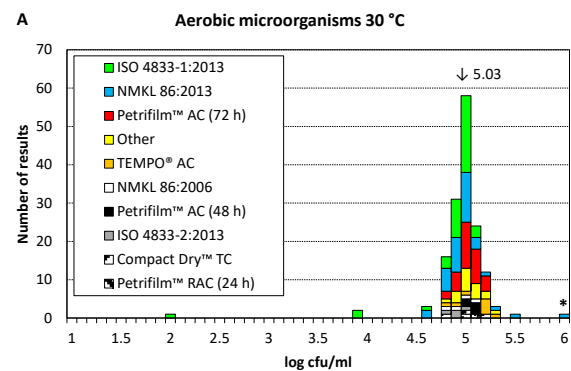
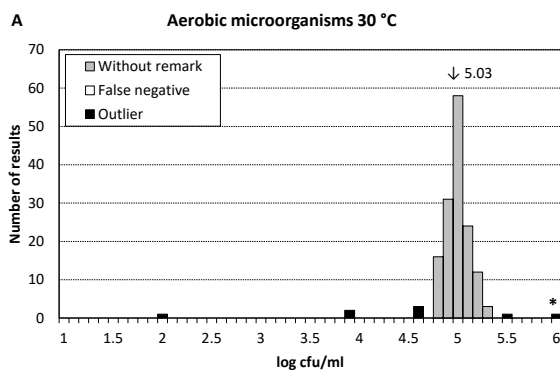
validation. For example, AOAC[®] prescribes incubation at 35 °C for 48 h while AFNOR prescribes 30 °C for either 48 h or 72 h, depending on which product that is analysed.

TEMPO[®] AC 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, at 30 °C, results from this method were slightly higher compared to results from other methods. Small differences like this are not uncommon for TEMPO[®] AC and can be considered normal.

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

Method	Sample A							Sample B							Sample C						
	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>
All results	152	144	5.03	0.13	0	6	2	151	143	5.01	0.12	0	5	3	151	141	5.07	0.15	0	6	4
ISO 4833-1:2013	40	36	5.02	0.08	0	4	0	40	37	5.00	0.12	0	3	0	40	36	5.02	0.11	0	3	1
NMKL 86:2013	37	33	5.02	0.12	0	2	2	37	36	4.94	0.13	0	0	1	36	33	5.02	0.12	0	1	2
Petrifilm™ AC (72 h)	32	32	5.07	0.10	0	0	0	32	31	5.09	0.09	0	1	0	34	34	5.10	0.12	0	0	0
Other	17	17	5.08	0.12	0	0	0	15	15	5.03	0.12	0	0	0	16	15	5.16	0.17	0	1	0
TEMPO [®] AC	8	8	5.22	0.17	0	0	0	8	6	5.01	0.17	0	0	2	8	8	5.18	0.20	0	0	0
NMKL 86:2006	4	4	-	-	0	0	0	5	4	-	-	0	1	0	5	4	-	-	0	1	0
Petrifilm™ AC (48 h)	5	5	5.15	0.06	0	0	0	5	5	5.03	0.09	0	0	0	3	3	-	-	0	0	0
ISO 4833-2:2013	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Compact Dry™ TC	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	2	-	-	0	0	1
Petrifilm™ RAC (24 h)	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).



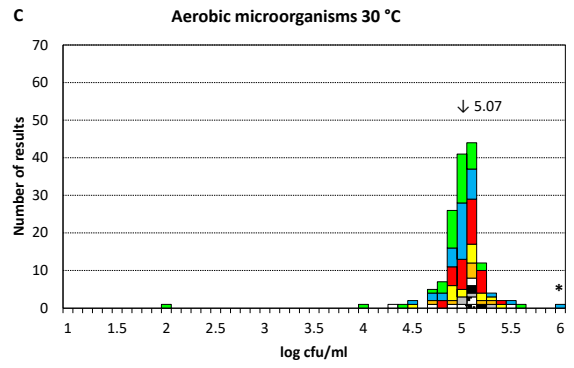
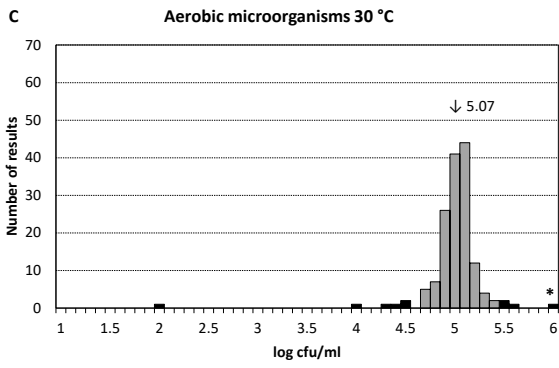
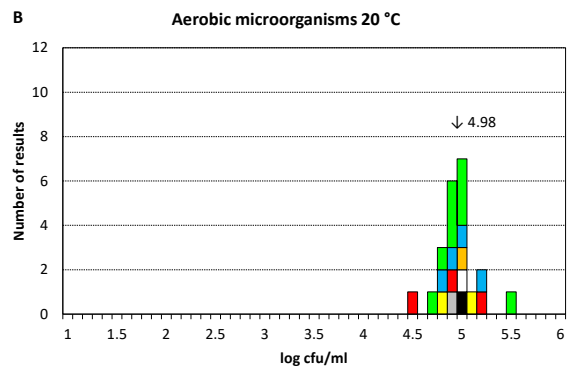
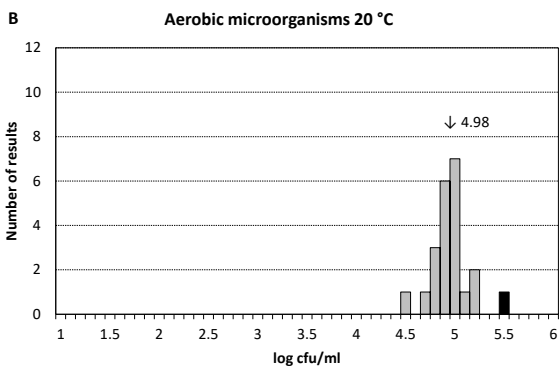
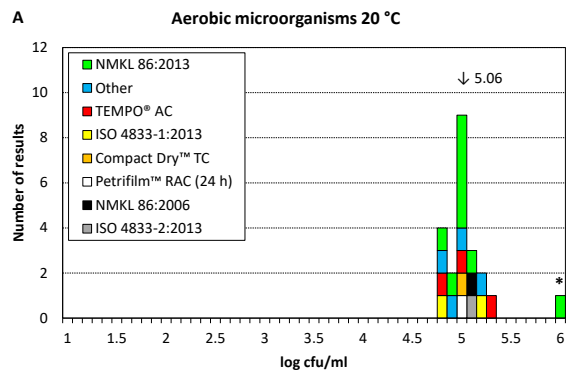
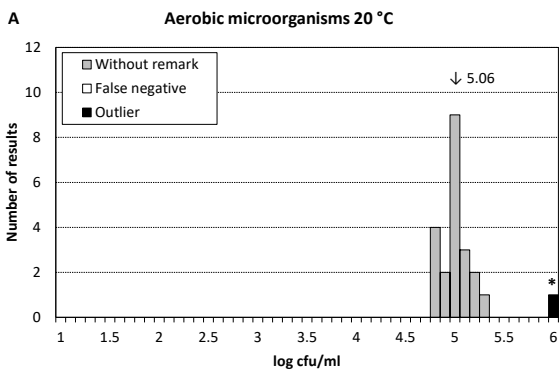


Figure 1. Results from analysis of aerobic microorganisms, 30 °C.

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

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	22	21	5.06	0.15	0	0	1	22	21	4.98	0.15	0	0	1	21	19	5.06	0.22	0	1	1
NMKL 86:2013	9	8	5.04	0.09	0	0	1	9	8	4.99	0.10	0	0	1	9	8	5.12	0.13	0	0	1
Other	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
TEMPO® AC	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
ISO 4833-1:2013	2	2	-	-	0	0	0	2	2	-	-	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
Petrifilm™ RAC (24 h)	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
NMKL 86:2006	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
ISO 4833-2:2013	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	0	1	0

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



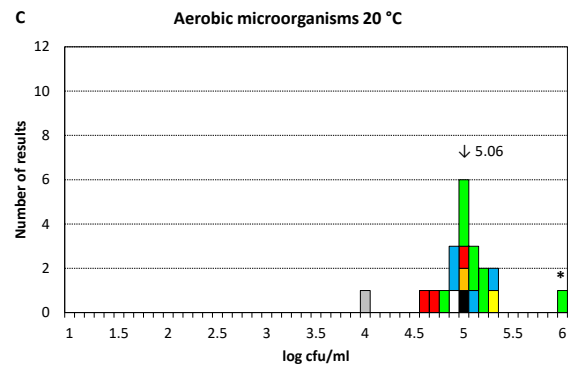
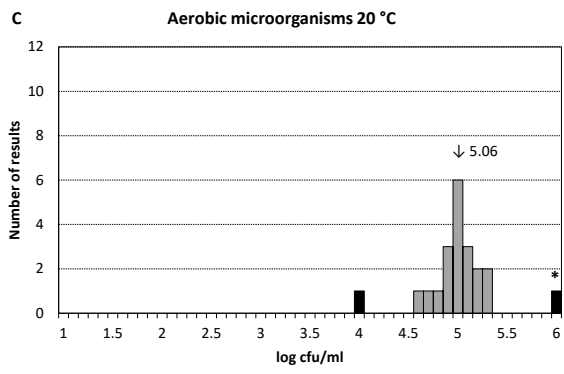


Figure 2. Results from analysis of aerobic microorganisms, 20 °C.

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. The latter may therefore be excluded if a catalase test is performed.

In total, 15 results were evaluated. All of these are considered acceptable.

Note: Even though the standard deviation (s_{PT}) is fairly low, the measurement uncertainty of the assigned value (u_{PT}) is not negligible. The evaluation of the results could therefore be affected, and Z-scores should be interpreted with caution.

Sample B

All three strains in the sample are capable of forming colonies on PCA. All three strains are catalase-positive.

In total, 14 results were evaluated. All of these are considered acceptable.

Note: Even though the standard deviation (s_{PT}) is fairly low, the measurement uncertainty of the assigned value (u_{PT}) is not negligible. The evaluation of the results could therefore be affected, and Z-scores should be interpreted with caution.

Sample C

All strains in the sample were target organisms and were present in similar concentrations. *B. cereus*, *K. pneumoniae* and *S. saprophyticus* are catalase-positive, whereas *E. faecalis* is catalase-negative. The latter may therefore be excluded if a catalase test is performed.

In total, 15 results were evaluated. All of these are considered acceptable.

Note: Even though the standard deviation (s_{PT}) is fairly low, the measurement uncertainty of the assigned value (u_{PT}) is not negligible. The evaluation of the results could therefore be affected, and Z-scores should be interpreted with caution.

General remarks

Only 15 results were reported, and the statistical analysis was therefore based on a somewhat limited dataset. The measurement uncertainties of the assigned values are therefore not negligible, and the results should be interpreted with some caution. Still, compared to historical PT results for this analysis, the results are well clustered, with distinct peaks, for all three samples. The standard deviations are also very low, compared to s_{PT} in previous PT rounds.

The only method specified by the participants was ISO 13559:2002 / IDF 153:2002. This was last reviewed by ISO in 2019 and remains current. The remaining participants reported “Other” method.

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.

Table 4. Results from analysis of contaminating microorganisms, 20 °C.

Method	Sample A							Sample B							Sample C							
	N	n	<i>m</i> _{PT}	<i>s</i> _{PT}	F	<	>	N	n	<i>m</i> _{PT}	<i>s</i> _{PT}	F	<	>	N	n	<i>m</i> _{PT}	<i>s</i> _{PT}	F	<	>	
All results	15	15	4.92	0.20	0	0	0	14	14	5.00	0.22	0	0	0	15	15	4.97	0.22	0	0	0	0
ISO 13559:2002 / IDF 153:2002	11	11	5.00	0.17	0	0	0	10	10	5.05	0.26	0	0	0	11	11	5.04	0.19	0	0	0	0
Other	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0	0

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

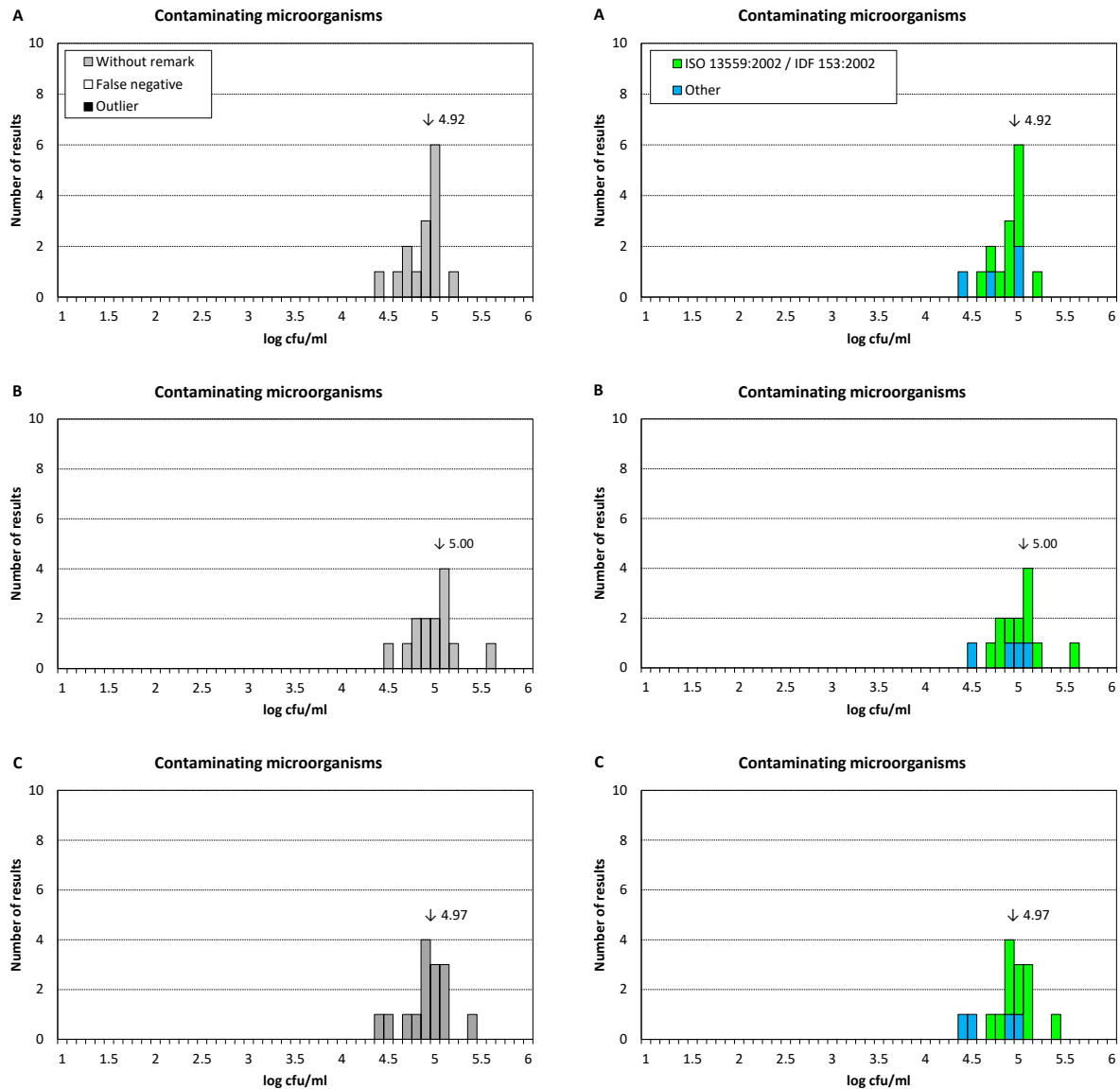


Figure 3. Results from analysis of contaminating microorganisms, 20 °C.

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, 132 results were evaluated. Four low and one high outliers were identified, as well as two false negative results.

Note: Five reported results were excluded from the evaluation, since they were not reported correctly.

Sample B

Both *E. coli* and *P. mirabilis* belong to Enterobacteriaceae. Both form colonies on VRBG and both are oxidase-negative.

In total, 132 results were evaluated. One low outlier was identified.

Note: Five reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

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

In total, 131 results were evaluated. One low outlier was identified, as well as four false negative results.

Note: Five reported results were excluded from the evaluation, since they were not reported correctly.

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.

The most common methods were NMKL 144:2005, a method with Petrifilm EB and ISO 21528-2:2017. ISO 21528-2:2017 was last reviewed by ISO in 2022 and remains current.

Most methods used by the participants stipulate a 37 °C incubation temperature. With Petrifilm EB, both 30 °C and 37 °C is possible to use. Here, two participants used the lower incubation temperature. One of these reported a false negative result for sample A, but it is difficult to determine if this was due to the incubation temperature or some other factor.

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	132	125	4.34	0.25	2	4	1	132	131	4.50	0.35	0	1	0	131	126	4.02	0.34	4	1	0
NMKL 144:2005	45	43	4.26	0.25	0	2	0	46	45	4.28	0.32	0	1	0	46	43	4.03	0.31	2	1	0
Petrifilm™ EB (37 °C)	37	37	4.48	0.17	0	0	0	37	37	4.76	0.16	0	0	0	37	37	4.09	0.33	0	0	0
ISO 21528-2:2017	25	23	4.35	0.22	0	1	1	24	24	4.35	0.32	0	0	0	24	24	3.98	0.36	0	0	0
Other	12	10	4.36	0.20	1	1	0	12	12	4.58	0.30	0	0	0	11	9	3.94	0.26	2	0	0
TEMPO® EB	6	6	4.40	0.28	0	0	0	6	6	4.72	0.48	0	0	0	6	6	3.98	0.43	0	0	0
Compact Dry™ ETB	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Petrifilm™ EB (30 °C)	2	1	-	-	1	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
BIOLOG-LM-278	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).

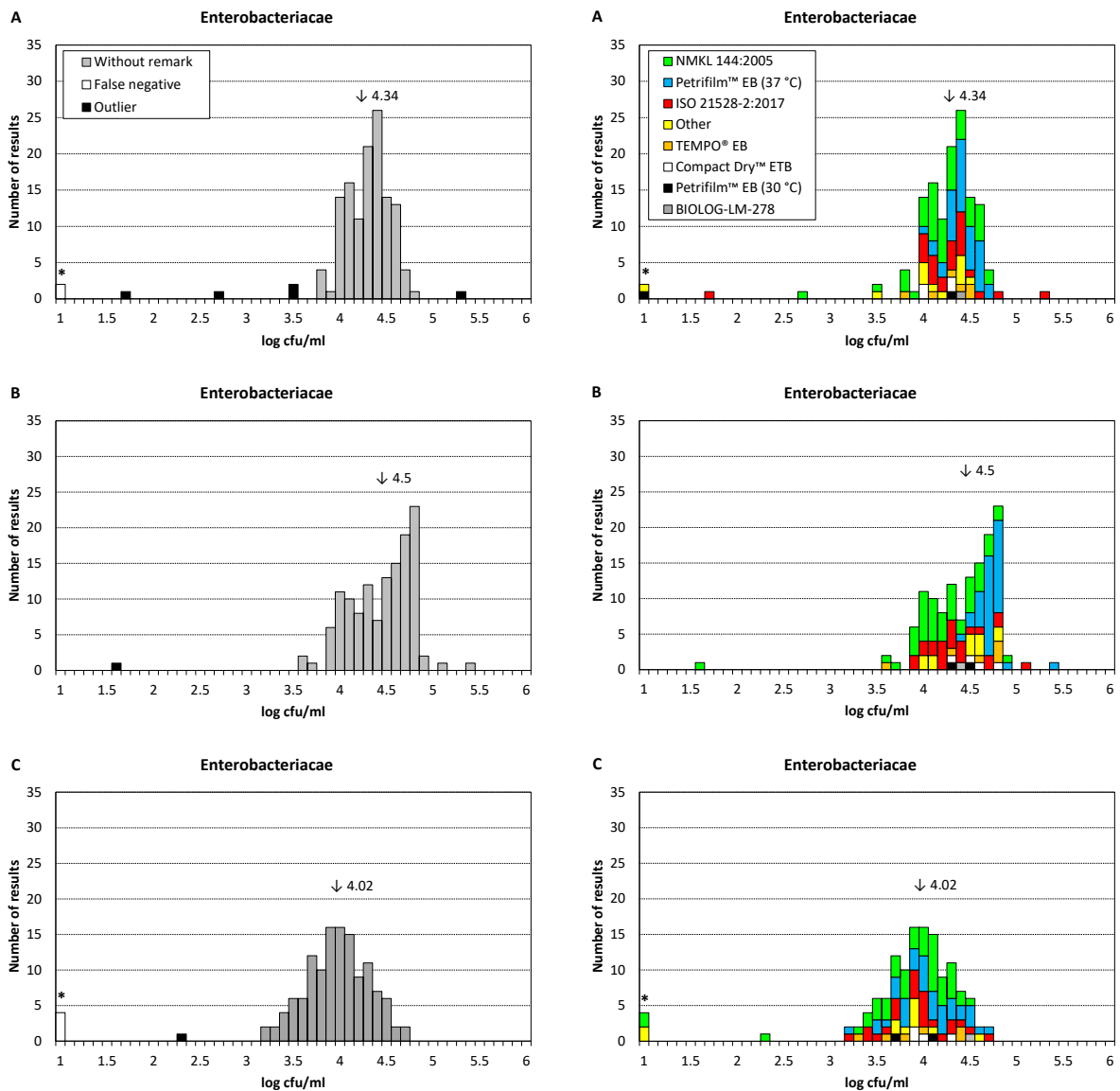


Figure 4. Results from analysis of Enterobacteriaceae.

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, 35 results were evaluated. All of the evaluated results are considered acceptable.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

For the analysis at 37 °C, 72 results were evaluated. Three false negative results were identified.

Note: Two reported results were excluded from the evaluation, since they were not reported correctly.

Sample B

The strain of *E. coli* (not identical to that in sample A) 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.

The strain of *P. mirabilis* also forms colonies on VRB. It is oxidase-negative but can be distinguished from coliform bacteria since it does not ferment lactose.

For the analysis at 30 °C, 36 results were evaluated. All of the evaluated results are considered acceptable.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

For the analysis at 37 °C, 72 results were evaluated. One low outlier was identified, as well as four false negative results.

Note: Three reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

K. pneumoniae 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 results were evaluated. All of the evaluated results are considered acceptable.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

For the analysis at 37 °C, 69 results were evaluated. One low outlier was identified, as well as two false negative results.

Note: Four reported results were excluded from the evaluation, since they were not reported correctly.

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 ISO 4832:2006 or NMKL 44:2004, which both use VRB as the primary medium. ISO 4832:2006 was last reviewed by ISO in 2021 and remains current. NMKL 44 was reviewed by NMKL in 2024 and remains current.

In addition to ISO 4832:2006 and NMKL 44:2004, at 37 °C, Petrifilm CC and Petrifilm EC/CC were used by many participants. Since all of the main methods are based on media with a similar composition, differences in results are 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. Participants may also perform 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. In this PT however, there was no apparent problem with identifying the target organisms, and the results from the different methods and media were similar. It could be noted though that, proportionally, fairly many false negative results were reported by participants that used Petrifilm REC.

Many methods were used by only a few participants each, meaning that the group “Other” is fairly large, in particular for 37 °C. Among the methods used by only a few participants were the MPN-based 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.

A few participants used methods/media that detect β-galactosidase and β-glucuronidase activity; RAPID'E.coli 2 and Compact Dry EC. 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.

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

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	35	35	4.28	0.30	0	0	0	36	36	4.21	0.31	0	0	0	38	38	3.98	0.33	0	0	0
ISO 4832:2006	17	17	4.15	0.34	0	0	0	18	18	4.17	0.32	0	0	0	19	19	3.90	0.32	0	0	0
NMKL 44:2004	11	11	4.23	0.27	0	0	0	10	10	4.17	0.31	0	0	0	10	10	4.01	0.28	0	0	0
Other	5	5	4.48	0.22	0	0	0	5	5	4.28	0.24	0	0	0	5	5	3.70	0.59	0	0	0
Petrifilm™ EC/CC	1	1	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Petrifilm™ CC	1	1	-	-	0	0	0	1	1	-	-	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).

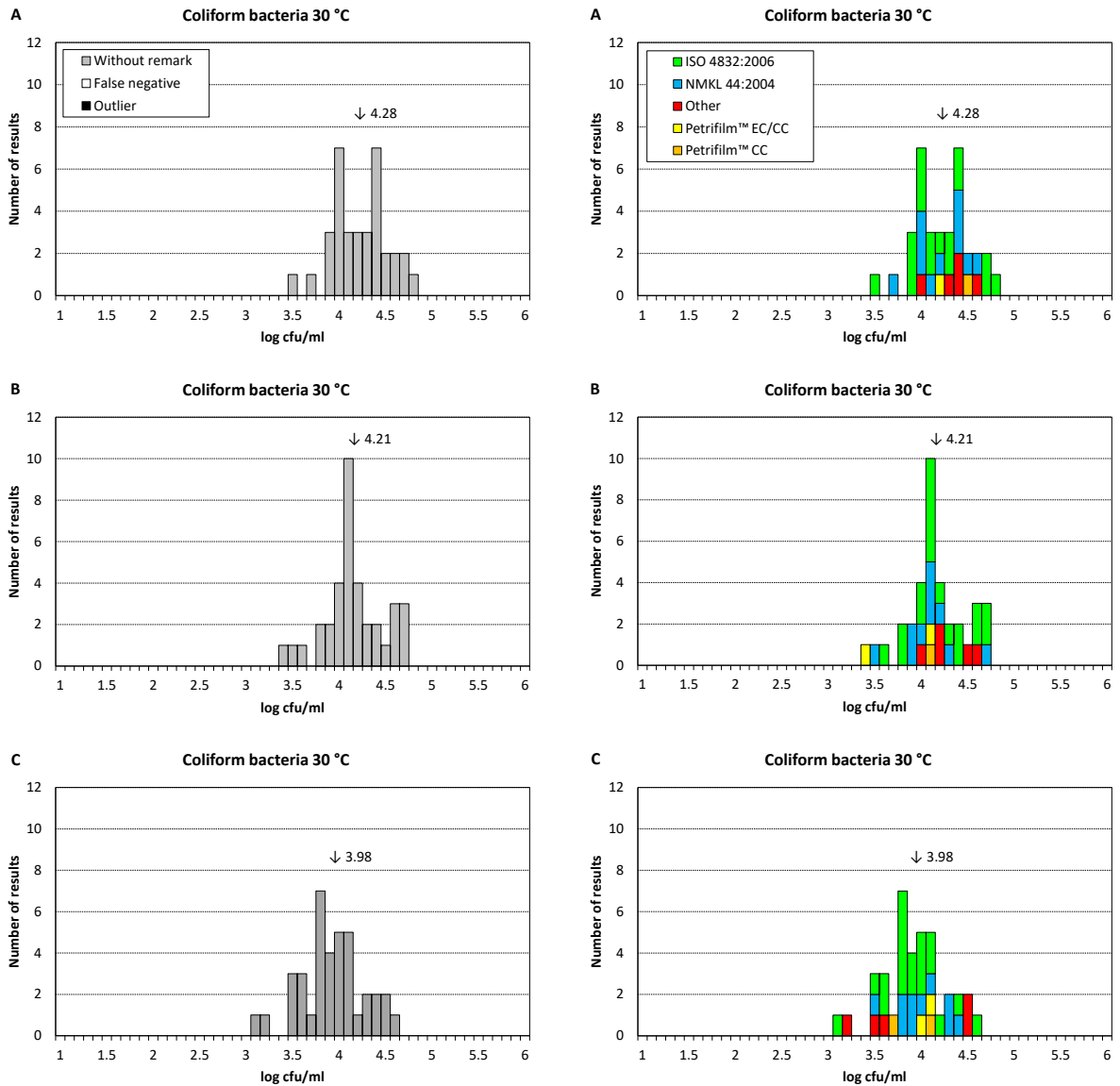


Figure 5. Results from analysis of coliform bacteria, 30 °C.

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

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	72	69	4.36	0.26	3	0	0		72	67	4.27	0.32	4	1	0		69	66	4.06	0.31	2	1	0	
Other	14	13	4.36	0.32	1	0	0		15	14	4.19	0.23	1	0	0		14	12	3.97	0.32	2	0	0	
NMKL 44:2004	14	14	4.46	0.30	0	0	0		14	14	4.29	0.36	0	0	0		14	14	4.03	0.29	0	0	0	
Petrifilm™ EC/CC	12	12	4.44	0.19	0	0	0		12	12	4.48	0.30	0	0	0		12	12	4.24	0.27	0	0	0	
Petrifilm™ CC	11	11	4.45	0.12	0	0	0		11	9	4.30	0.35	2	0	0		10	10	3.97	0.34	0	0	0	
ISO 4832:2006	9	9	4.30	0.36	0	0	0		9	8	4.13	0.21	0	1	0		8	8	4.22	0.26	0	0	0	
Petrifilm™ REC	5	3	-	-	2	0	0		4	3	-	-	1	0	0		3	3	-	-	0	0	0	
ISO 4831:2006 (MPN)	3	3	-	-	0	0	0		3	3	-	-	0	0	0		3	2	-	-	0	1	0	
RAPID ¹ E.coli2	2	2	-	-	0	0	0		2	2	-	-	0	0	0		2	2	-	-	0	0	0	
Compact Dry™ EC	1	1	-	-	0	0	0		1	1	-	-	0	0	0		2	2	-	-	0	0	0	
TEMPO® CC	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).

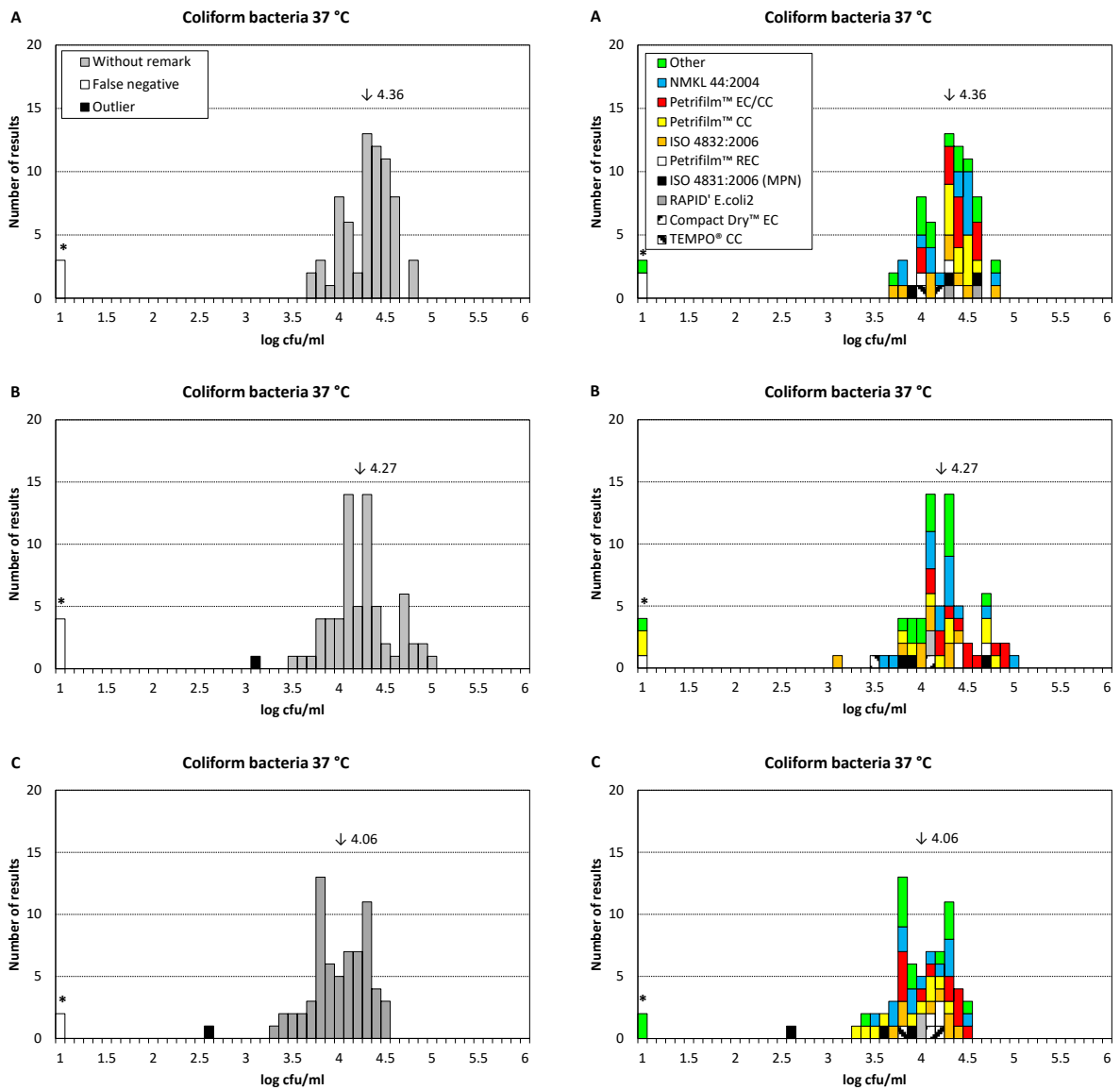


Figure 6. Results from analysis of coliform bacteria, 37 °C.

Thermotolerant coliform bacteria and *Escherichia coli*

Sample A

The strain of *E. coli* was target organism for both analyses. On VRB, it forms typical red colonies surrounded by a bile salt precipitation zone. The strain is positive for indole production and β -glucuronidase activity and produces gas in LTLSB.

For thermotolerant coliform bacteria, 24 results were evaluated. All of the evaluated results are considered acceptable.

Note: Two reported results were excluded from the evaluation, since they were not reported correctly.

For *E. coli*, 103 results were evaluated. Three low outliers were identified.

Note: Eight reported results were excluded from the evaluation, since they were not reported correctly.

Sample B

The strain of *E. coli* (not identical to that in sample A) was target organism for both analyses. On VRB, it forms typical red colonies surrounded by a bile salt precipitation zone. The strain is positive for indole production and β -glucuronidase activity, and produces gas in LTLSB.

At the Swedish Food Agency, additional small colonies (presumably *P. mirabilis*) were detected on TSA/VRB at 44 °C. These colonies were smaller than *E. coli* and could easily be distinguished since they were negative for indole production and β -glucuronidase activity, and did not produce gas in LTLSB.

For thermotolerant coliform bacteria, 25 results were evaluated. All of the evaluated results are considered acceptable.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

For *E. coli*, 104 results were evaluated. Two low and one high outliers were identified, as well as two false negative results.

Note: Seven reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

The strain of *K. pneumoniae* was target organisms for thermotolerant coliform bacteria. On VRB, it forms typical red colonies surrounded by a bile salt precipitation zone. The strain produces gas in LTLSB, but is negative for indole production and β -glucuronidase activity. No *E. coli* was present in the sample.

For thermotolerant coliform bacteria, 23 results were evaluated. One low outlier was identified, as well as two false negative results.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

For *E. coli*, 105 results were evaluated. Two false positive results were reported.

Note: Two reported results were excluded from the evaluation, since they were not reported correctly.

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 enzyme β -glucuronidase.

NMKL 125:2005 was the most common method for both analyses. It is based on VRB and describes the analysis of both thermotolerant coliform bacteria and of *E. coli*. A new version of this was published in 2024. The new version, NMKL 125:2024, includes determination of β -glucuronidase positive *E. coli*.

For *E. coli*, other common methods were Petrifilm SEC and Petrifilm EC/CC. Both of these are based on substrates that facilitate detection of β -glucuronidase, and *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. Petrifilm EC/CC was used by participants with both 24 h and 48 h incubation. Based on the available data, it is not possible to determine if the different incubation times had an effect on the outcome.

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 somewhat lower results compared to participants that use other media. This was seen both for samples A and B 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 2024 and remains current.

For *E. coli*, the mean value for the MPN-based TEMPO EC is often somewhat higher compared to other methods/media. This was the case both for samples A and B in this PT round.

For *E. coli*, many methods were used by only a few participants, meaning that the group “Other” is fairly large. Among the methods used by only a few participants were NMKL 96:2009 and RAPID' E.coli2.

Table 8. Results from analysis of thermotolerant coliform bacteria.

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	24	24	4.43	0.21	0	0	0	25	25	4.27	0.23	0	0	0	23	20	4.08	0.41	2	1	0	0
NMKL 125:2005	15	15	4.49	0.12	0	0	0	15	15	4.23	0.21	0	0	0	14	14	4.23	0.33	0	0	0	0
Petrifilm™ CC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0	0
NMKL 96:2009	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0	0
ISO 16649-2:2001*	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	1	0	0	0
Petrifilm™ REC	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	1	0	0	0
Other	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0	0
ISO 7251:2005*	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	0	-	-	0	1	0	0
Compact Dry™ EC	0	0	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0	0
ISO 4832:2006	1	1	-	-	0	0	0	1	1	-	-	0	0	0	0	0	-	-	0	0	0	0

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

* Method primarily aimed at detection of *E. coli*.

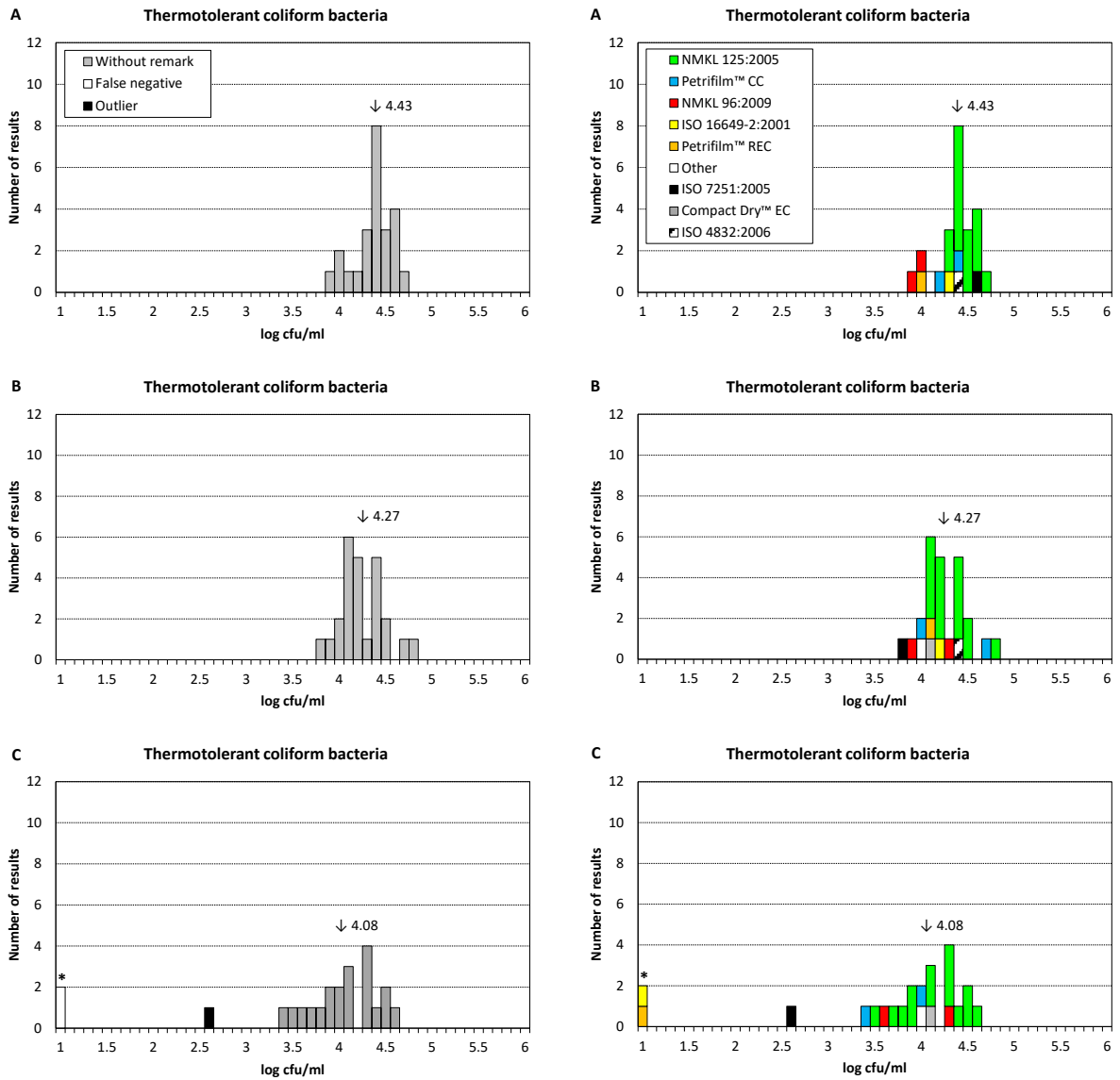


Figure 7. Results from analysis of thermotolerant coliform bacteria.

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

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	103	100	4.40	0.22	0	3	0	104	99	4.22	0.21	2	2	1	105	103	-	-	2	-	-
NMKL 125:2005	24	24	4.50	0.19	0	0	0	24	22	4.25	0.15	1	0	1	25	24	-	-	1	-	-
Petrifilm™ SEC	20	19	4.48	0.23	0	1	0	20	19	4.23	0.17	1	0	0	20	20	-	-	0	-	-
Petrifilm™ EC/CC (48 h)	15	15	4.48	0.15	0	0	0	15	15	4.20	0.21	0	0	0	14	14	-	-	0	-	-
Other	13	12	4.30	0.34	0	1	0	13	12	4.17	0.26	0	1	0	12	12	-	-	0	-	-
ISO 16649-2:2001	12	11	4.28	0.21	0	1	0	13	13	4.06	0.25	0	0	0	11	11	-	-	0	-	-
TEMPO® EC	7	7	4.50	0.27	0	0	0	7	7	4.41	0.16	0	0	0	7	7	-	-	0	-	-
Petrifilm™ EC/CC (24 h)	6	6	4.38	0.15	0	0	0	6	5	4.25	0.09	0	1	0	6	5	-	-	1	-	-
Petrifilm™ REC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	4	4	-	-	0	-	-
ISO 7251:2005	2	2	-	-	0	0	0	2	2	-	-	0	0	0	4	4	-	-	0	-	-
Compact Dry™ EC	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-

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

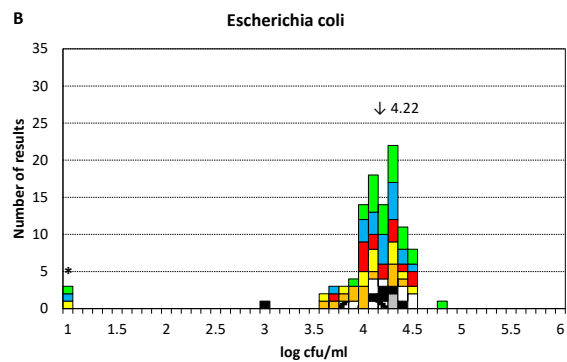
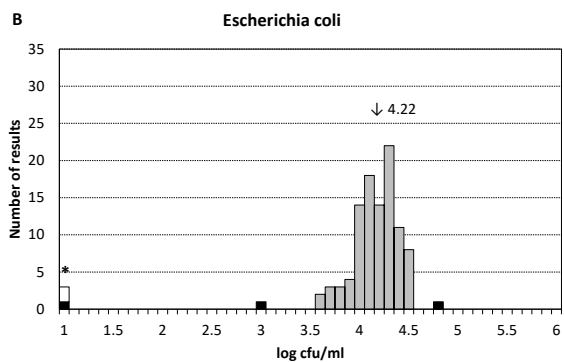
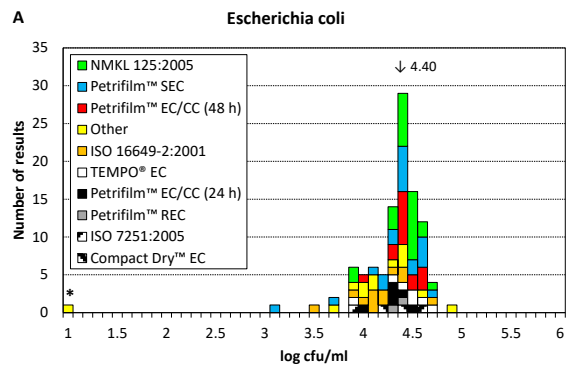
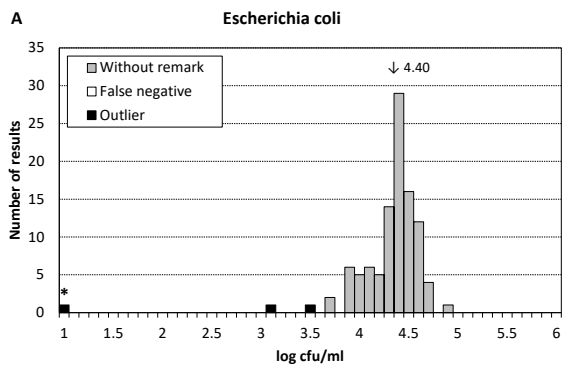


Figure 8. Results from analysis of *Escherichia coli*.

Presumptive *Bacillus cereus*

Sample A

The strain of *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, 87 results were evaluated. One low outlier was identified.

Note: Four reported results were excluded from the evaluation, since they were not reported correctly.

Sample B

No *B. cereus* was present in the sample. In the quality control at the Swedish Food Agency, only atypical colonies, without a zone of haemolysis, formed on BcsA. Similarly on BA, none of the colonies showed the typical haemolysis of *B. cereus*.

In total, 84 results were evaluated. Two false positive results were reported.

Note: Six reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

The strain of *B. cereus* (identical to that in sample A) 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, 87 results were evaluated. One low outlier was identified.

Note: Four reported results were excluded from the evaluation, since they were not reported correctly.

General remarks

B. cereus is a Gram-positive bacterium, which on BA forms large, irregular grey colonies, surrounded by a distinct zone of haemolysis. On the selective medium BcsA, presumptive *B. cereus* instead 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, usually surrounded by a zone of precipitation, again as a consequence of lecithinase activity.

Many participants followed an NMKL method, either NMKL 67:2021 or the older NMKL 67:2010. They mainly differ in the order of the selective and non-selective medium. No apparent differences in the results between the two methods could be seen in this PT.

ISO 7932:2004 was also used by many participants. It was last reviewed by ISO in 2021 and remains current. An amendment with optional test was published in 2020.

A few participants used Compact Dry X-BC which contains chromogenic and selective agents in that 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.

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

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	87	86	4.38	0.24	0	1	0	84	82	-	-	2	-	-	87	86	4.49	0.23	0	1	0
ISO 7932:2004	21	20	4.40	0.19	0	1	0	19	19	-	-	0	-	-	21	20	4.46	0.23	0	1	0
NMKL 67:2021	19	19	4.38	0.24	0	0	0	22	22	-	-	0	-	-	20	20	4.54	0.18	0	0	0
NMKL 67:2010	17	17	4.50	0.29	0	0	0	17	15	-	-	2	-	-	16	16	4.64	0.28	0	0	0
Other	16	16	4.31	0.21	0	0	0	12	12	-	-	0	-	-	16	16	4.39	0.19	0	0	0
Compact Dry™ X-BC	5	5	4.32	0.32	0	0	0	5	5	-	-	0	-	-	5	5	4.37	0.18	0	0	0
TEMPO® BC	3	3	-	-	0	0	0	4	4	-	-	0	-	-	3	3	-	-	0	0	0
ISO 7932:2004 / Amd 1:2020	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
BACARA®	2	2	-	-	0	0	0	1	1	-	-	0	-	-	2	2	-	-	0	0	0
BIOLOG-LM-401	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).

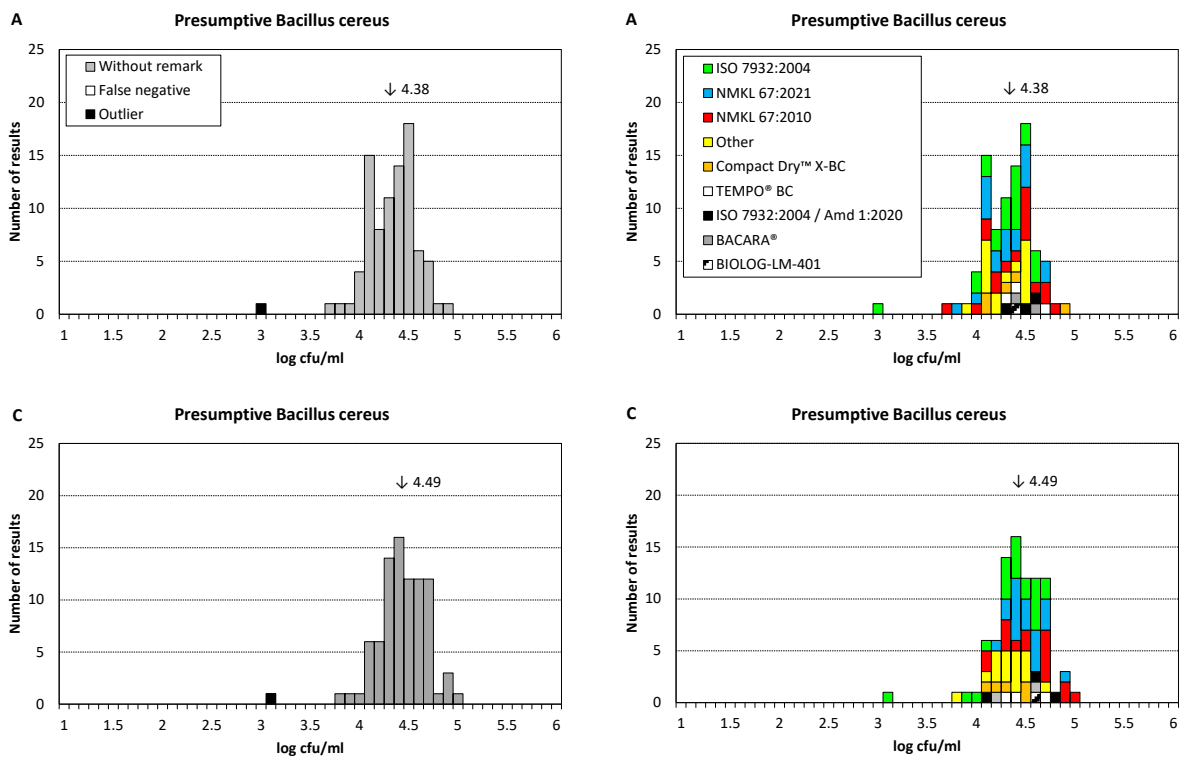


Figure 9. Results from analysis of presumptive *Bacillus cereus*.

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, 83 results were evaluated. Three low and one high outliers were identified, as well as three false negative results.

Note: Three reported results were excluded from the evaluation, since they were not reported correctly.

Sample B

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

In total, 83 results were evaluated. Six low outliers were identified, as well as one false negative result.

Note: Three reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

No target organism was present in the samples. The coagulase-negative strain of *S. saprophyticus* is false-positive for the analysis. On RPFA, it forms atypical colonies without a coagulase zone.

In total, 82 results were evaluated. Two false positive results were reported.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

General remarks

The majority of the participants followed methods based on incubation on BP or 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. When using BP, a confirmation is typically performed based on coagulase activity, for example a tube coagulase test or the use of RPFA as a secondary medium. With RPFA, the coagulase activity is tested directly in the medium.

In comparison, Petrifilm Staph Express is based on a modified Baird-Parker agar. It contains a chromogenic indicator that causes *S. aureus* to form red/purple colonies. The associated Petrifilm Staph Express Disk facilitates 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.

With NMKL 66:2009, 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. Amendments with clarifications to ISO 6888-1:2021 and ISO 6888-2:2021 were published in 2023.

Three participants used a method with TEMPO STA. With this method, one low outlier was reported for sample A. This has been seen previously for this strain, and likely reflects difficulties with detecting this particular strain of *S. aureus* with TEMPO STA.

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

Method	Sample A							Sample B							Sample C						
	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>
All results	83	76	4.47	0.14	3	3	1	83	76	4.56	0.11	1	6	0	84	82	-	-	2	-	-
NMKL 66:2009 (BP)	23	22	4.50	0.13	0	0	1	22	22	4.56	0.10	0	0	0	24	24	-	-	0	-	-
Petrifilm™ Staph	15	15	4.46	0.11	0	0	0	15	14	4.55	0.07	0	1	0	15	15	-	-	0	-	-
Other	15	12	4.51	0.11	1	2	0	14	10	4.59	0.11	1	3	0	15	14	-	-	1	-	-
ISO 6888-1:2021 (BP)	13	13	4.53	0.22	0	0	0	14	13	4.65	0.09	0	1	0	11	11	-	-	0	-	-
ISO 6888-2:2021 (RPFA)	5	4	-	-	1	0	0	5	5	4.52	0.08	0	0	0	5	5	-	-	0	-	-
ISO 6888-2:2021 / Amd 1:2023 (RPFA)	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	-	-
NMKL 66:2009 (RPFA)	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	-	-
Compact Dry™ X-SA	3	2	-	-	1	0	0	3	3	-	-	0	0	0	3	2	-	-	1	-	-
TEMPO® STA	2	1	-	-	0	1	0	3	2	-	-	0	1	0	3	3	-	-	0	-	-
ISO 6888-1:2021 / Amd 1:2023 (BP)	1	1	-	-	0	0	0	1	1	-	-	0	0	0	2	2	-	-	0	-	-

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

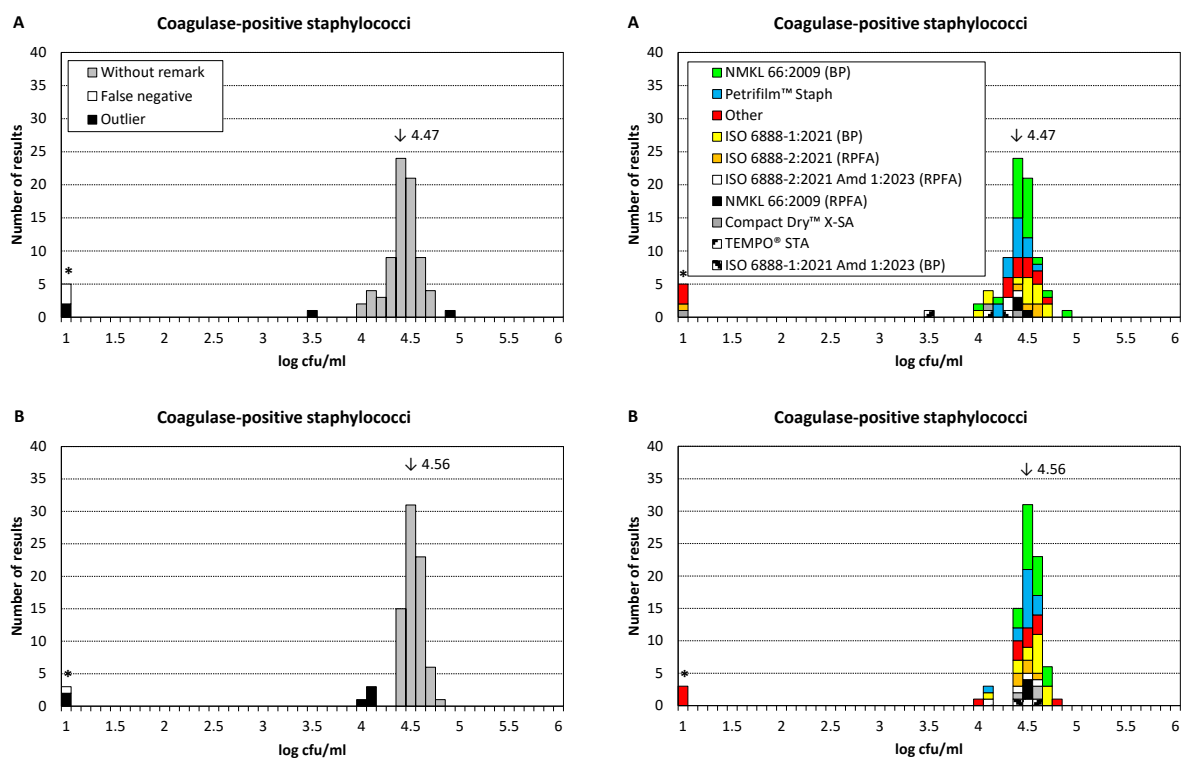


Figure 10. Results from analysis of coagulase-positive staphylococci.

Enterococci

Sample A

The strain of *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, 50 results were evaluated. Four low outliers were identified.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

Sample B

No target organism was present in the sample. In the quality control at the Swedish Food Agency, atypical red colonies formed on ENT. On BEAA, no typical black colour could be seen.

In total, 49 results were evaluated. Four false positive results were reported.

Note: Two reported results were excluded from the evaluation, since they were not reported correctly.

Sample C

The strain of *E. faecalis* was target organism. On ENT, it forms typical dark red colonies. On BEA, a black colour is typically seen after both 2 h and 24 h incubation.

In total, 51 results were evaluated. One low and one high outliers were identified, as well as one false negative result.

Note: One reported result was excluded from the evaluation, since it was not reported correctly.

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. Pre-incubation on TSA can be preferable if the presence of stressed enterococci is expected. 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. Similar to BEA, Compact Dry ETC detects β -glucosidase activity, but is instead based on the substrate X-Gluc. On this medium, enterococci therefore form blue colonies.

A few participants followed the drinking water method ISO 7899-2:2000. This was last reviewed by ISO in 2021 and remains current. ISO 27205:2010/IDF 149:2010, which is a method for fermented milk products, was last reviewed by ISO in 2020 and remains current.

Table 12. Results from analysis of enterococci.

Method	Sample A							Sample B							Sample C						
	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>s</i> _{PT}	<i>F</i>	<	>
All results	50	46	4.43	0.18	0	4	0	49	45	-	-	4	-	-	51	48	4.41	0.10	1	1	1
NMKL 68:2011	31	29	4.43	0.16	0	2	0	31	28	-	-	3	-	-	30	29	4.41	0.10	0	0	1
Other	9	9	4.44	0.18	0	0	0	8	8	-	-	0	-	-	10	9	4.43	0.09	0	1	0
ISO 7899-2:2000	4	4	-	-	0	0	0	4	3	-	-	1	-	-	5	5	4.41	0.14	0	0	0
Compact Dry™ ETC	3	2	-	-	0	1	0	3	3	-	-	0	-	-	3	2	-	-	1	0	0
ISO 27205:2010 / IDF 149:2010	3	2	-	-	0	1	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0

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

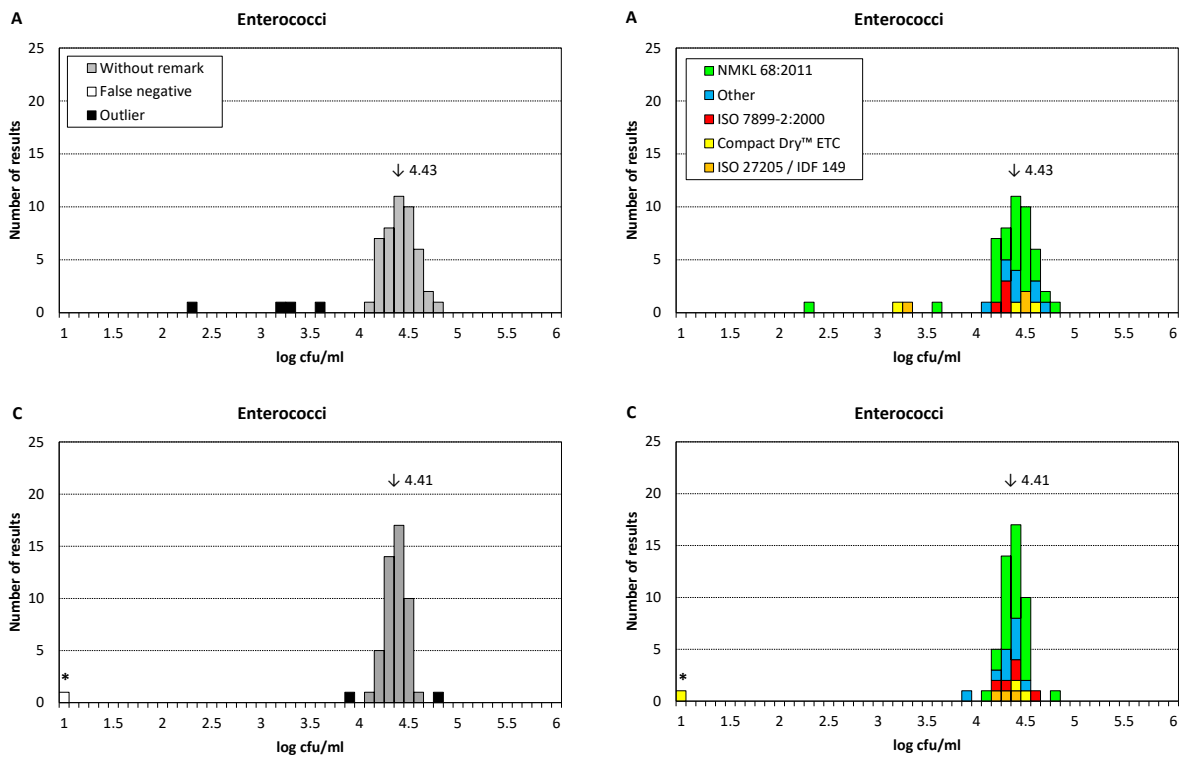


Figure 11. Results from analysis of enterococci.

Gram-negative bacteria in pasteurised milk and cream

Sample A

E. coli is Gram-negative. It forms colonies on VRBG.

In total, eight results were evaluated. All reported a correct positive result.

Sample B

E. coli and *P. mirabilis* are Gram-negative.

In total, eight results were evaluated. All reported a correct positive result.

Sample C

K. pneumoniae is Gram-negative.

In total, eight results were evaluated. One false negative result was reported.

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.

The majority of the participants followed NMKL 192:2011. One participant followed a company-specific method and one followed ISO 21528-1:2017. These methods are all based on incubation on VRBG, on which the detection of Gram-negative is not expected to be problematic.

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

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	8	8	-	-	0	-	-	8	8	-	-	0	-	-	9	8	-	-	1	-	-
NMKL 192:2011	6	6	-	-	0	-	-	6	6	-	-	0	-	-	6	5	-	-	1	-	-
Other	2	2	-	-	0	-	-	2	2	-	-	0	-	-	2	2	-	-	0	-	-
ISO 21528-2:2017	0	0	-	-	0	-	-	0	0	-	-	0	-	-	1	1	-	-	0	-	-

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

Outcome of the results of individual participants - assessment

Reporting and evaluation of results

The results of all participants are listed in Appendix 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: <https://laboratory.livsmedelsverket.se>

Box plots and numbers of deviating results for each participant

Box plots are based on the z -scores listed in Appendix 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.

The different parts of a box plot are shown in figure 12.

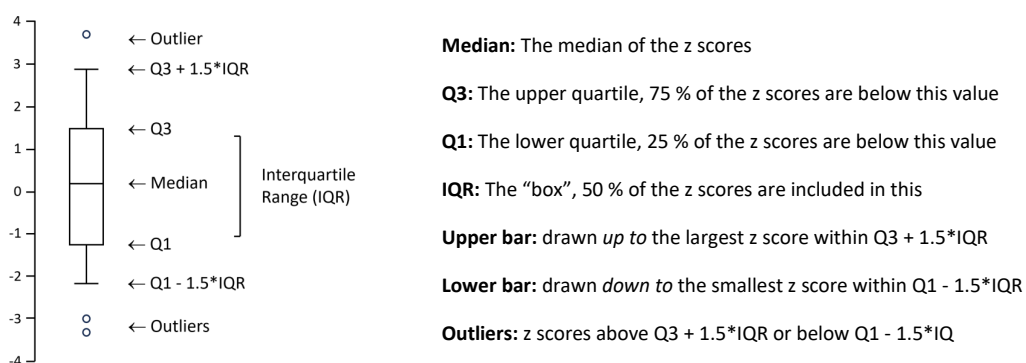
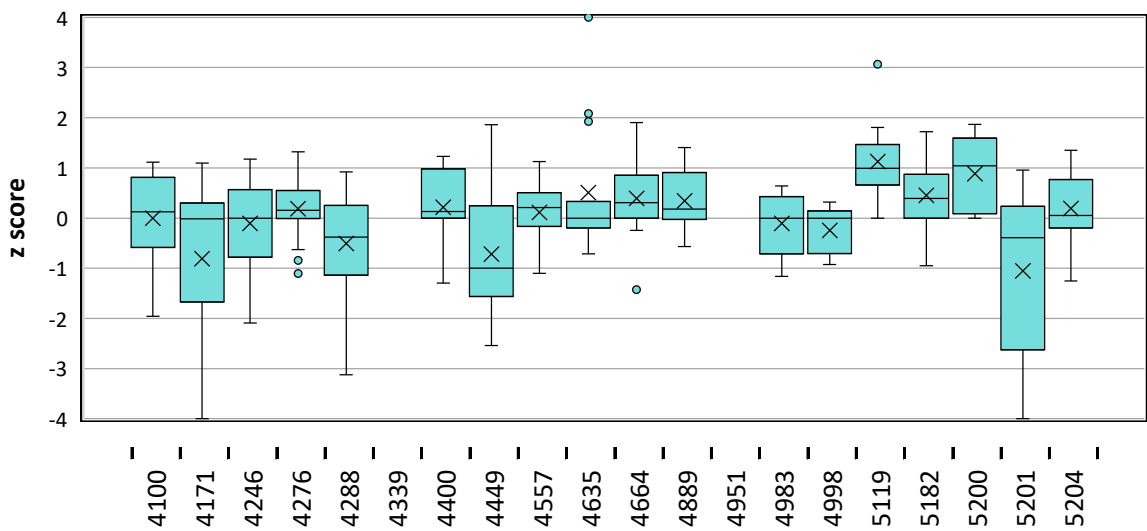
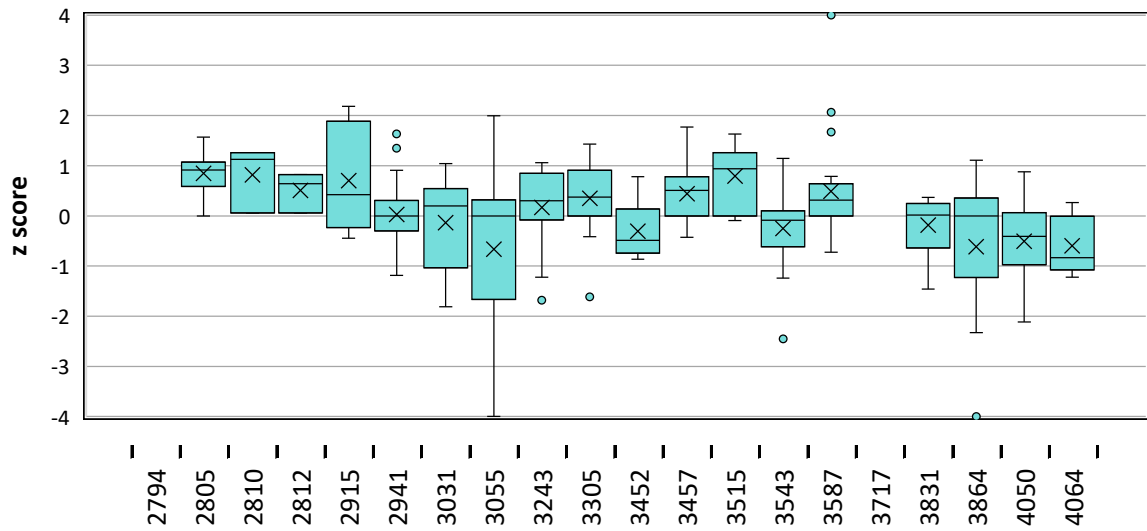
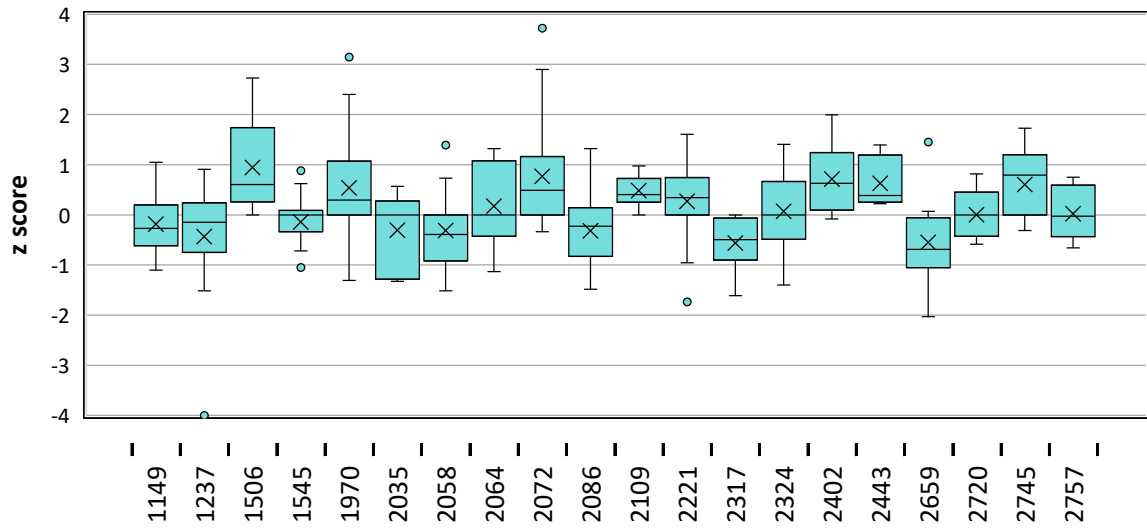
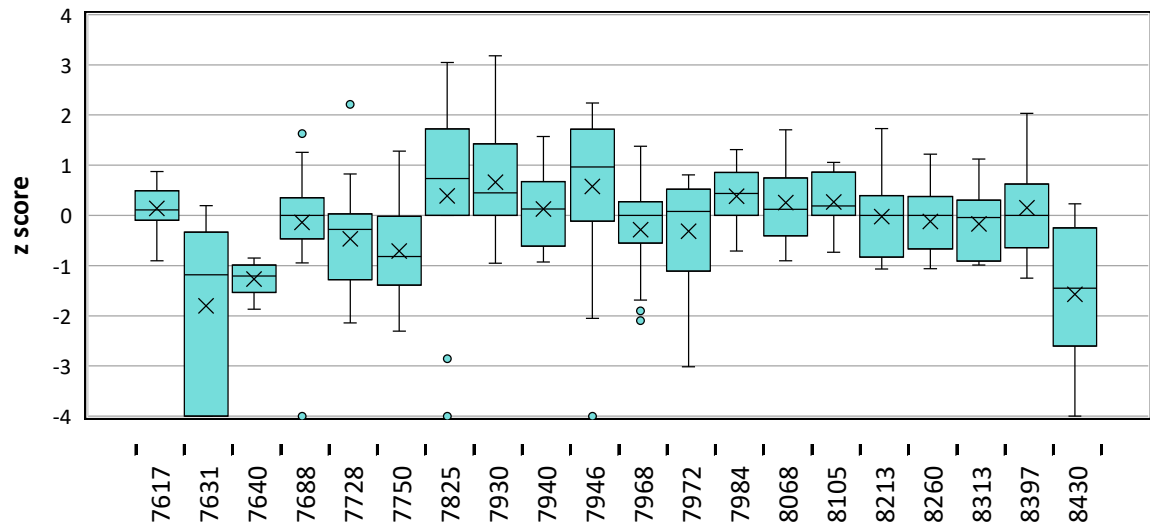
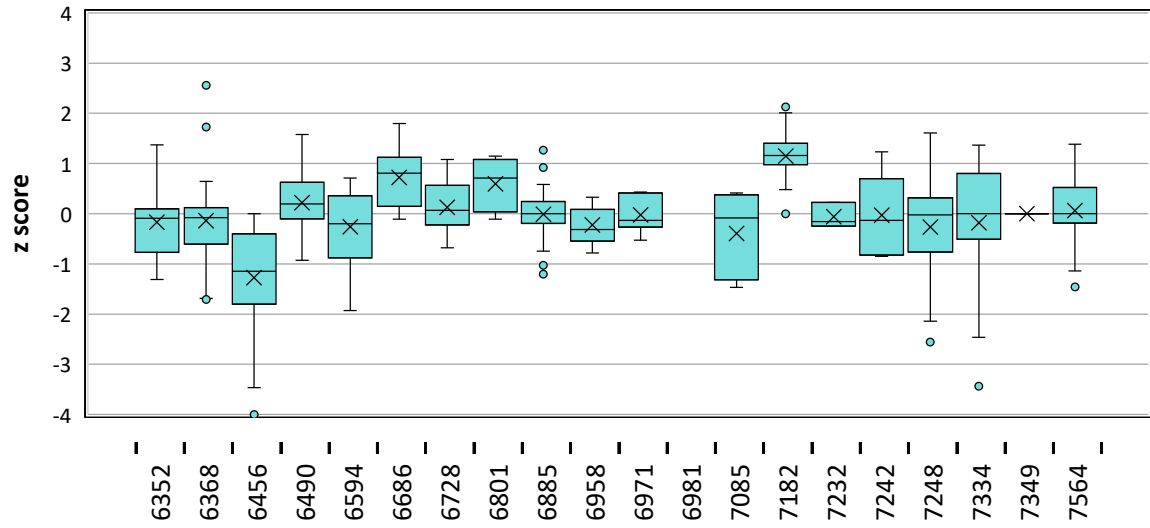
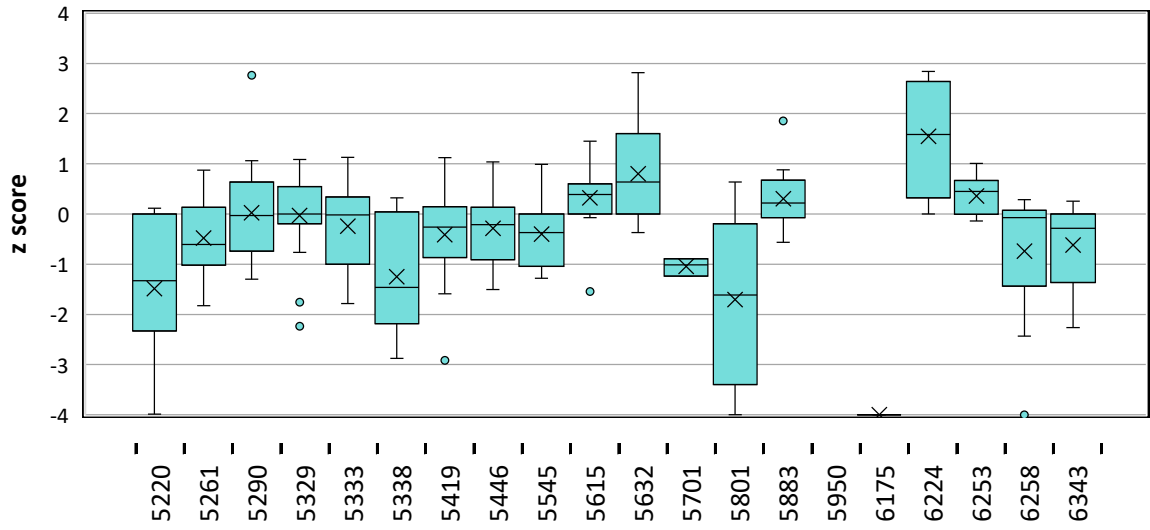
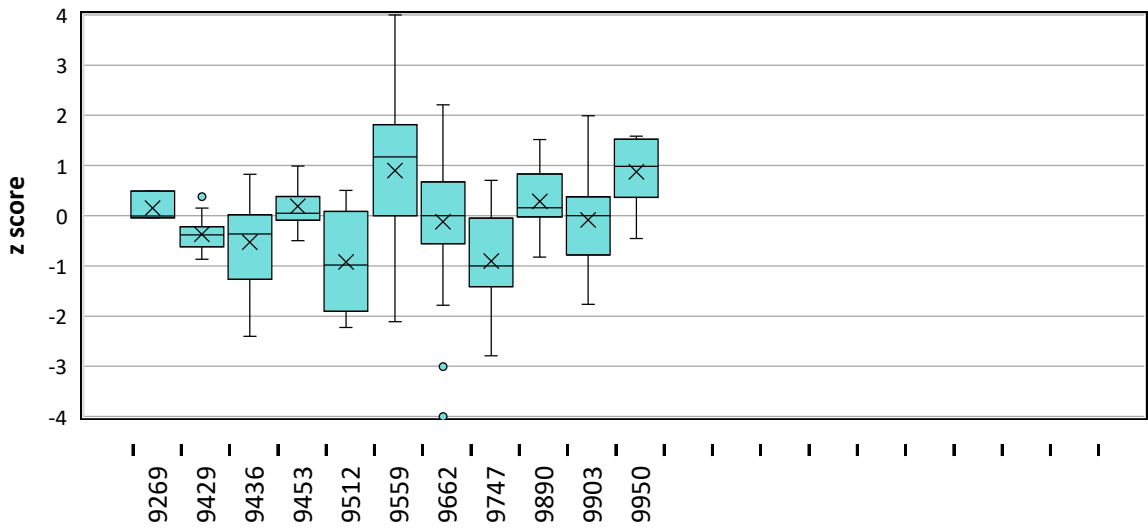
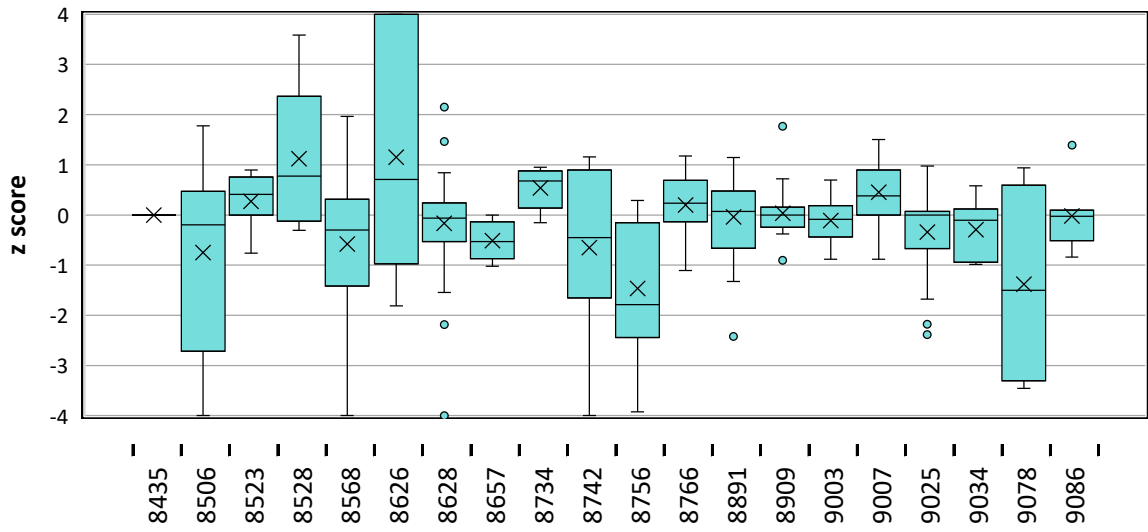


Figure 12. Schematic explanation of a box plot.







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 table 14.

Table 14. Microorganisms and approximate concentrations in the samples.

Sample	Microorganism	Strain			
		SLV no. ¹	Origin	Reference ²	log ₁₀ cfu ml ⁻¹
A	<i>Bacillus cereus</i>	SLV-202	Hen	CCUG 45144	5.1
	<i>Escherichia coli</i>	SLV-085	Water	-	4.6
	<i>Enterococcus durans</i>	SLV-078	Fresh meat	CCUG 44816	4.4
	<i>Staphylococcus aureus</i>	SLV-185	Chicken	CCUG 48090	4.5
B	<i>Escherichia coli</i>	SLV-477	Cheese	CCUG 43601	4.4
	<i>Proteus mirabilis</i>	SLV-180	Horse meat	CCUG 48088	5.1
	<i>Staphylococcus aureus</i>	SLV-280	Egg	-	4.7
C	<i>Bacillus cereus</i>	SLV-202	Hen	CCUG 45144	4.6
	<i>Enterococcus faecalis</i>	SLV-051	-	CCUG 45101	4.5
	<i>Klebsiella pneumoniae</i>	SLV-186	Vegetarian kebab	CCUG 45102	4.0
	<i>Staphylococcus saprophyticus</i>	SLV-013	-	CCUG 45100	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; SMI: Public Health Agency of Sweden.

Quality control of the samples

Quality control and evaluation of sample homogeneity is performed on 10 randomly chosen vials in conjunction with manufacture, or on 5 vials if an “old” batch of samples is used. Homogeneity of a test material is approved if, for each analysis, the p value of a one-way analysis of variance (ANOVA) fulfils the criterion $p \geq 0.05$. If the ANOVA yields $p < 0.05$, the PT test item batch is still considered homogenous, if $s_{bb} < s_R/3$, where:

s_{bb} : the between-vial standard deviation from the ANOVA

s_R : the expected laboratory variation, generally assumed to be 0.25 for the Food scheme.

See the Scheme protocol [2] for more information regarding the evaluation of homogeneity.

Table 15. Concentration mean (m), between-vial variation (s_{bb}) and p 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 ²		
	m	s_{bb}	p	m	s_{bb}	p	m	s_{bb}	p
Aerobic microorganisms, 30 °C NMKL method no. 86:2013	4.92	0.05	0.03	4.97	0.05	0.16	5.10	0.04	0.08
Aerobic microorganisms, 20 °C NMKL method no. 86:2013	5.02	0.00	0.80	5.05	0.03	0.16	5.10	0.04	0.12
Contaminating microorganisms ISO method no. 13559:2002 IDF method no. 153:2002	4.95	0.00	0.74	4.80	0.02	0.29	5.02	0.05	0.05
Enterobacteriaceae NMKL method no. 144:2005	4.31	0.00	0.66	4.67	0.03	0.02	4.02	0.00	0.50
Coliform bacteria, 30 °C NMKL method no. 44:2004	4.22	0.04	0.33	4.19	0.06	0.01	3.99	0.10	0.29
Coliform bacteria, 37 °C NMKL method no. 44:2004	4.25	0.08	0.15	4.19	0.04	0.10	3.93	0.00	0.94
Thermotolerant coliform bacteria NMKL method no. 125:2005	4.55	0.09	0.11	4.36	0.04	0.22	3.99	0.07	0.08
Escherichia coli NMKL method no. 125:2005	4.55	0.09	0.11	4.36	0.04	0.22	-	-	-
Presumptive Bacillus cereus NMKL method no. 67:2021	4.53	0.00	0.68	-	-	-	4.63	0.00	0.87
Coagulase-positive staphylococci NMKL method no. 66:2009	4.56	0.00	0.71	4.64	0.05	0.10	-	-	-
Enterococci NMKL method no. 68:2011	4.45	0.04	0.20	-	-	-	4.50	0.01	0.48
Gram-negative bacteria in milk and cream NMKL method no. 192:2011	Pos.	-	-	Pos.	-	-	Pos.	-	-

– No target organism or no value

¹ $n = 5$ vials analysed in duplicate

² $n = 10$ 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. 2024. 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.

Appendix 1. Results of the participating laboratories

Lab no.	Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorganisms			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 milk products															
	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													
9436-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-									
9453-1	5.02	5	5.08	-	-	-	5.01	5.05	5.03	4.43	4.6	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
9453-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
9512-1	4.813	4.799	4.74	-	-	-	-	-	-	4.264	4.672	4.004	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
9512-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
9559-1	5.56	5.26	5.33	5.21	5.26	5.36	-	-	-	3.82	4.99	3.99	-	-	-	3.84	5.06	3.99	-	-	-	4.79	4.38	0	4.82	0	4.79	4.72	4.84	0	-	-	-	-	-	-	-	-	-	-	-	Pos	Pos	Pos					
9559-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
9662-1	5.02	4.8	5.15	4.87	4.8	5.16	-	-	-	4.7	4.58	4.16	4.76	4.16	4.23	4.32	4.12	4.3	-	-	-	4.29	3.6	0	4.41	0	5	4.39	4.07	4.8	4.68	0	4.44	-	-	-	-	-	-	-	-	-	-	-					
9662-2	4.91	5.11	5.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
9747-1	4.84	4.68	4.92	-	-	-	-	-	-	4	4.03	3.99	-	-	-	-	-	-	-	-	-	-	-	-	4.55	0	4.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
9747-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
9890-1	5.05	4.92	5.09	-	-	-	-	-	-	4.62	4.6	4.41	-	-	-	4.38	4.76	4.2	-	-	-	4.38	4.36	0	4.57	0	4.71	4.36	4.51	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9890-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9903-1	5.05	4.98	4.96	-	-	-	-	-	-	4.42	4.49	3.42	-	-	-	-	-	-	-	-	-	-	-	-	4.53	3.99	0	4.15	0	4.95	4.57	4.76	0	4.3	0	4.26	-	-	-	-	-	-	-	-	-	-	-	-	
9903-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9950-1	5.23	5.13	5.21	-	-	-	-	-	-	4.23	4.72	4.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9950-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
N	152	151	151	22	22	21	15	14	15	132	132	131	35	36	38	72	72	69	24	25	23	103	104	105	87	84	87	83	83	84	50	49	51	8	8	9	-	-	-	-	-	-	-	-	-	-	-		
n	144	143	141	21	21	19	15	14	15	125	131	126	35	36	38	69	67	66	24	25	20	100	99	103	86	82	86	76	76	82	46	45	48	8	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-
Min	2.02	1.93	2.01	4.81	4.58	4.06	4.49	4.58	4.49	0	1.60	0	3.57	3.45	3.18	0	0	0	3.97	3.88	0	1.00	0	0	3.00	0	3.18	0	0	0	2.38	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Max	7.64	7.66	7.69	6.38	5.50	6.19	5.20	5.61	5.44	5.38	5.49	4.73	4.80	4.76	4.61	4.90	5.06	4.59	4.76	4.89	4.60	4.97	4.88	3.84	4.91	4.93	5.00	4.91	4.84	4.80	4.87	4.49	4.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Med	5.04	5.02	5.08	5.04	4.99	5.07	5.00	5.03	4.99	4.37	4.57	4.03	4.26	4.17	3.93	4.40	4.28	4.07	4.45	4.21	4.13	4.45	4.23	0	4.41	0	4.49	4.49	4.57	0	4.43	0	4.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
m_{PT}	5.03	5.01	5.07	5.06	4.98	5.06	4.92	5.00	4.97	4.34	4.50	4.02	4.28	4.21	3.98	4.36	4.27	4.06	4.43	4.27	4.08	4.40	4.22	-	4.38	-	4.49	4.47	4.56	-	4.43	-	4.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
s_{PT}	0.13	0.12	0.15	0.15	0.15	0.22	0.20	0.22	0.22	0.25	0.35	0.34	0.30	0.31	0.33	0.26	0.32	0.31	0.21	0.23	0.41	0.22	0.21	-	0.24	-	0.23	0.14	0.11	-	0.18	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
u_{PT}	0.013	0.012	0.015	0.039	0.039	0.061	0.066	0.075	0.072	0.027	0.038	0.038	0.064	0.065	0.067	0.040	0.049	0.047	0.054	0.058	0.111	0.027	0.026	-	0.032	-	0.031	0.019	0.015	-	0.033	-	0.018	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
F+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
F-	0	0	0	0	0	0	0	0	0	2	0	4	0	0	0	3	4	2	0	0	2	0	2	0	0	0	0	3	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1
<	6	5	6	0	0	1	0	0	0	4	1	1	0	0	0	0	1	1	0	0	1	3	2	0	1	0	1	3	6	0	4	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
>	2	3	4	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Lower	4.63	4.65	4.63	4.62	4.54	4.40	4.30	4.33	4.30	3.60	3.45	3.00	3.37	3.27	2.99	3.57	3.31	3.13	3.79	3.58	2.86	3.74	3.60	0	3.66	0	3.79	4.06	4.24	0	3.87	0	4.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
Upper	5.43	5.37	5.50	5.50	5.43	5.73	5.53	5.67	5.63	5.09	5.55	5.04	5.19	5.14	4.96	5.15	5.24	4.99	5.07	4.96	5.30	5.07	4.84	0	5.11	0	5.18	4.89	4.89	0	4.98	0	4.73	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

- False positive or false negative
- Outside the acceptance limits
- Results "larger than" are not evaluated
- The parameter is not evaluated
- The result not evaluated
- u_{PT} > 0,3 s_{PT} and/or > 20 % outliers and/or fewer than 12 evaluated results

N = number of reported results
 n = results without annotation
 Min = lowest reported result
 Max = highest reported result
 Med = median value
 m_{PT} = assigned value
 s_{PT} = standard deviation
 u_{PT} = measurement uncertainty
 F+ = false positive
 F- = false negative
 < = low outlier
 > = high outlier
 Lower = lowest accepted value
 Upper = highest accepted value

Appendix 2. Z-scores of all participants

Lab no.	Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorganisms			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 milk products		
	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
5200-1	1.868	1.043	1.320																									0.173								
5200-2																												0								
5201-1	-4.000	-0.949	-4.000							0.394	0.180	0.408										-0.287	0.955	0	-2.528	-1.485	-2.917	-0.497	0							
5201-2																																				
5204-1	0.901	0.725	0.985				-0.272	-0.092	0.021	-0.172	-0.621	0.525	-0.174	-0.959	0.317	-1.171	-0.382	0.040	0.670	0.560	0.968	0.841	1.003	0	0.536	0	0.833	-1.256	-0.404	0	-0.965	0	-0.913			
5204-2	0.599	-0.363	0.711							1.242	1.039	1.348										0.751	0.858	0				0.260	0.060	0						
5220-1	-3.176	-3.292	-1.818							-1.303	-1.707	0.114										-3.984	-2.330	0				-1.112	-1.424	0						
5220-2																												-0.968	-1.331	0						
5261-1	-1.620	-0.681	-1.825							0.543	-0.306	0.273						0.874				-1.161	-0.606	0	-0.810	0	-0.871									
5261-2																																				
5290-1	0.675	1.059	2.763							0.596	-0.992	-0.797	0.189	-0.029	-1.295			-0.301				0.119	0.810		-1.037	0	-0.626	-0.029	-0.682							
5290-2																																				
5329-1	0.629	-0.614	0.889							-1.756	0.687	-0.051										-2.235	-0.765	0	0.515	0	0.404	0.505	1.089	0	-0.042		0.226			
5329-2																																				
5333-1	-0.080	-0.447	-0.246				0.608	0.933	-0.205	-0.778	-1.135	-1.562	-0.437	0.773	-1.204	0.340	-1.314	-1.220				0.210	0.037	0	-1.575	0	-1.785	1.126	0.894	0	-1.073	0	0.342			
5333-2	0.524	0.557	0.233							-0.778	0.695	-1.503																-0.534	-0.033	0						
5338-1	-1.364	-2.873	-1.955							-0.051	-1.564	0.320																								
5338-2																																				
5419-1							-0.027	-0.849	-0.746	-1.222	-1.221	-0.709										-1.098	-0.639		0.205		-1.013	-2.917	-0.868	0	-0.259	0	-0.527			
5419-2										0.676	1.124	0.143										0.435	0.327		0			0.430	0	-0.856	0	-1.589				
5446-1	-0.911	0.557	0.164							-0.980	1.039	-0.210				-1.133	-0.195	-0.639				-1.504	0.133	0				-0.318	-0.311	0						
5446-2																																				
5545-1	-1.062	-1.116	-1.135							-1.278	-0.768														-0.582	0	-0.927		0.987	0	-0.042	0	0.148			
5545-2	-0.156	0.557	-0.998																																	
5615-1	0.599	0.557	0.780							0.353	0.524	0.261			0.408	-0.075	1.452											-1.545	0.709	0						
5615-2																																				
5632-1	2.034	2.816	1.600							0.636	0.867	1.701										0.435	0.375	0	0.701	0	-0.369	0.909	0.338	0						
5632-2																																				
5701-1	-1.235	-0.890	-1.012																																	
5701-2																																				
5801-1	-3.251	-3.543	-4.000							0.636	-0.392	-1.620																-1.575	0	-1.614						
5801-2																																				
5883-1	-0.307	0.139	0.780							0.636	-0.563	0.878				1.852	0.302	0.848				-0.287	0.375	0	-0.002	0	0.490	-0.318	0.616	0						
5883-2																																				
5950-1																																				
5950-2																																				
6175-1	-4.000	-4.000	-4.000							-4.000	-4.000	-4.000																								
6175-2																																				
6224-1	1.581	2.649	2.626							1.363	2.841	0.202													2.192	0	0.447									
6224-2																																				
6253-1										-0.010	1.010	0.525	0.683	-0.093	0.530							0.796	0.375	0					0.609	0	-0.141					
6253-2																																				
6258-1	0.146	-0.447	-0.041										-0.075	-2.434	0.286							-0.106	-4.000	0												
6258-2																																				
6343-1	-1.356	-1.384	-2.229							-0.992	0.252	-0.212				0.144	-0.366	-0.173				-0.025	-0.325	0	-0.242	0	-0.493	-2.260	-1.452	0						
6343-2																																				
6352-1	-0.080	-0.782	-0.724							-0.455	-0.764	-1.238				0.605	-0.226	-0.994				0.390		0	1.322	0	-1.227	-0.101	0	1.369	0	1.017				
6352-2																												-0.499	0	-1.313						
6368-1	-0.080	-1.284	-0.383	-0.264	-0.971	-0.110				-0.899	-1.707	0.643				-0.945	-1.687	0.557	0.201	-0.436	0.575	0.300	-0.253	0	-0.002	0	-0.626	0.043	-0.589	0	-0.313	0	-0.527			
6368-2																																				
6456-1										-1.061	-1.364	-0.533	-1.261	-1.215	-0.352	-1.927	-1.438					2.554	1.728	0	0.577	0	-0.712	-2.484	-3.463	0	-4.000	0	-1.300			
6456-2															-0.322													-0.679	-0.960	0						
6490-1	0.071	0.474	-0.930							0.596	0.810	-0.415				0.491	1.576	-0.574							0.784	0	0.704	0.332	0.245	0	-0.422		0.148			
6490-2																																				
6594-1	-0.760	0.223	0.711													-1.133	-1.003	-1.931																		
6594-2																0.491	-0.257	-0.251																		
6686-1	0.373	0.808	0.780	0.145	0.652	0.878				1.242	1.124	1.4																								

Appendix 2. Z-scores of all participants

Lab no.	Aerobic microorganisms 30 °C			Aerobic microorganisms 20 °C			Contaminating microorganisms			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 milk products								
	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						
9436-2																						0.345	-0.446	0																		
9453-1	-0.080	-0.112	0.096				0.462	0.220	0.291	0.353	0.295	-0.063													0.991	0	-0.111	0.982	-0.497	0	0.935	0	-0.334									
9453-2																																										
9512-1	-1.643	-1.794	-2.229							-0.317	0.501	-0.051																														
9512-2																																										
9559-1	3.997	2.063	1.805	1.032	1.870	1.327				-2.111	1.410	-0.092				-1.965	2.446	-0.218				1.743	0.761	0	1.819	0	1.305	1.776	2.563	0				0	0	0						
9559-2																																										
9662-1	-0.080	-1.786	0.575	-1.287	-1.241	0.429				1.444	0.238	0.408	1.573	-0.157	0.773	-0.151	-0.475	0.784				-0.512	-3.006	0	0.122	0	2.206	-0.607	-4.000		1.369	0	0.245									
9662-2	-0.911	0.808	-0.314																																							
9747-1	-1.439	-2.790	-0.998							-1.384	-1.336	-0.092													0.701	0	-0.798															
9747-2																																										
9890-1	0.146	-0.782	0.164							1.121	0.295	1.143				0.076	1.514	0.460				-0.106	0.665	0	0.784	0	0.962	-0.823	-0.497	0												
9890-2																																										
9903-1	0.146	-0.280	-0.724							0.313	-0.020	-1.767										0.570	-1.122	0	-0.954	0	1.992	0.693	1.821	0	-0.693	0	-1.493									
9903-2																																										
9950-1	1.505	0.976	0.985							-0.455	0.638	1.584																														
9950-2																																										

- $|z| \geq 3,0$ ("Unacceptable" or "Action")
- $2,0 < |z| < 3,0$ ("Warning")
- The parameter is not evaluated
- The result 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 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: www.livsmedelsverket.se/en/PT-micro

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