

Proficiency testing Drinking water Microbiology

March 2023

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Abbreviations

Media

ACTA	Actinomycete Isolation Agar (SS 028212)
DRBC	Dikloran Rose-Bengal chloramphenicol agar
CCA	Chromogenic Coliform Agar (EN ISO 9308-1:2014)
Colilert	Colilert™ Quanti-Tray™ (IDEXX Inc.; EN ISO 9308-2:2014)
LES	m-Endo Agar LES (SS 028167)
LTSB	Lactose tryptone lauryl sulphate broth (SS 028167)
m-FC	m-FC Agar (SS 028167)
ME	Malt Extract agar
PCA	Plate count agar
R2A	Reasoner's 2 Agar (Standard Methods, 9215 Heterotrophic Plate Count)
RBCC	Rose Bengal Agar with both chlortetracycline and chloramphenicol (SS 028192)
TSC	Tryptose Sulfite Cycloserine agar (EN ISO 14189:2016)
YEA	Yeast extract Agar (EN ISO 6222:1999)

Other abbreviations

MF	Membrane filter (method)
MPN	Most Probable Number (quantification based on statistical distributions)
ISO	International Organization for Standardization
EN	European standard from "Comité Européen de Normalisation" (CEN)
DS, NS, SFS, SS	National standards from Denmark, Norway, Finland and Sweden
SLV	Livsmedelsverket/Swedish Food Agency, Sweden

Analyses in this PT round

Quantitative analyses

Coliform bacteria

Suspected thermotolerant coliform bacteria (not assessed)

Escherichia coli

Clostridium perfringens

Actinomycetes

Moulds

Yeast

Culturable microorganisms, 72 hours incubation at 22 °C

Slow-growing bacteria, 7 days incubation at 22 °C

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 square root 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 coefficient of variation (*CV*) 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 *CV* 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.

Coefficient of variation

The coefficient of variation (*CV*) is a relative measure and is calculated as:

$$CV = 100 \times \frac{s_{PT}}{m_{PT}}$$

The CV for square root transformed results is given as a measure of dispersion. When the dispersion is <10 % it is regarded as very small, 10–20 % as small, 20–30 % as medium, 30–40 % as large and >40 % as very large.

Measurement uncertainty for the assigned value

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:

$$u_{PT} < 0,3s_{PT}$$

In annex 1 the relative standard uncertainty (u_{rel}) of m_{PT} is also provided.

$$u_{rel,mPT}(\%) = 100 \times \frac{s_{PT}}{\sqrt{n} \cdot m_{PT}}$$

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 acceptable result (false results and outliers excluded)
- m_{PT} assigned value, robust mean value in cfu/MPN 100 ml^{-1} or cfu ml^{-1} , re-transformed to the cfu scale
- Med median in cfu 100 ml^{-1}
- CV coefficient of variation in percent
- F number of false positive or false negative results
- < number of low outliers
- > number of high outliers

Figure legends

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

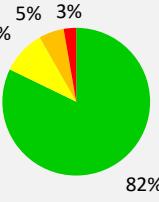
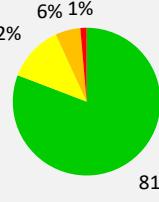
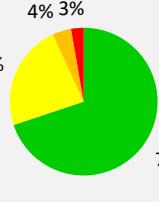
Results

General outcome

Samples were sent to 73 participants; 36 in Sweden, 36 in European countries, and one outside of Europe. Of the 73 participants that reported results, 34 (47 %) provided at least one result that received an annotation.

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												
Microorganisms	<i>Escherichia coli</i> <i>Aeromonas caviae</i> <i>Candida glabrata</i> <i>Phialophora fastigiata</i> <i>Stenotrophomonas maltophilia</i>			<i>Klebsiella oxytoca</i> <i>Citrobacter freundii</i> <i>Clostridium perfringens</i> <i>Streptomyces</i> species <i>Staphylococcus saprophyticus</i>			<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Clostridium bifermentans</i> <i>Macrococcus caseolyticus</i> <i>Sphingomonas</i> species <i>Streptomyces</i> species					
Analysis	Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Coliform bacteria (MF)	<i>E. coli</i> (<i>A. caviae</i>)	50	0	1	<i>K. oxytoca</i> <i>C. freundii</i>	50	0	3	<i>E. coli</i> <i>K. pneumoniae</i>	51	0	2
Susp. thermotol. coliform bact. (MF)	<i>E. coli</i>	25	-	-	(<i>K. oxytoca</i>)	25	-	-	<i>E. coli</i> <i>K. pneumoniae</i>	25	-	-
<i>E. coli</i> (MF)	<i>E. coli</i>	51	0	3	(<i>K. oxytoca</i>)	51	2	0	<i>E. coli</i> (<i>K. pneumoniae</i>)	51	0	2
Coliform bacteria (MPN)	<i>E. coli</i>	47	0	2	<i>K. oxytoca</i> <i>C. freundii</i>	47	1	0	<i>E. coli</i> <i>K. pneumoniae</i>	46	0	1
<i>E. coli</i> (MPN)	<i>E. coli</i>	47	0	2	-	47	0	0	<i>E. coli</i>	47	0	2
Presumptive <i>C. perfringens</i>	-	41	2	0	<i>C. perfringens</i>	41	2	2	<i>C. bifermentans</i>	41	2	2
<i>C. perfringens</i>	-	26	3	0	<i>C. perfringens</i>	26	2	1	(<i>C. bifermentans</i>)	26	10	0
Moulds	<i>P. fastigiata</i>	36	1	2	-	35	2	0	-	35	2	0
Yeast	<i>C. glabrata</i>	36	1	3	-	35	3	0	-	35	1	0
Actinomycetes	-	25	0	0	<i>Streptomyces</i> sp.	25	0	1	<i>Streptomyces</i> sp.	24	3	0
Culturable microorganisms 22 °C, 3 days	<i>S. maltophilia</i> <i>E. coli</i> <i>A. caviae</i>	69	0	2	<i>S. saprophyticus</i>	69	0	1	<i>S. cohnii</i> <i>E. coli</i> <i>K. pneumoniae</i>	69	0	2
Slow-growing bacteria	<i>S. maltophilia</i> <i>E. coli</i> <i>A. caviae</i>	41	-	-	<i>S. saprophyticus</i>	41	-	-	<i>Sphingomonas</i> sp. <i>S. cohnii</i> <i>E. coli</i> <i>K. pneumonia</i>	41	0	2

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

 The results are not evaluated

Coliform bacteria

Sample A

The strain of *E. coli* was target organism. The oxidase-positive strain of *A. caviae* was present as a false-positive organism for the analysis.

In total, 50 participants reported results for membrane filtration (MF) methods. One high outlier was reported.

For most probable number (MPN) methods, 47 participants reported results. One high and one low outlier were reported.

Sample B

The strains of *K. oxytoca* and *C. freundii* were target organisms. Both strains form typical colonies with a metallic sheen on m-Endo Agar LES (LES) and pink colonies on Chromocult Coliform Agar (CCA).

On CCA, *S. saprophyticus* may form small atypical pink colonies that should not be included in the result.

The strains of *K. oxytoca* and *C. freundii* both possess the enzyme β -galactosidase and are detected as coliform bacteria with ColilertTM/ColilertTM-18.

In total, 50 participants reported results for MF methods. Three high outliers were reported.

For MPN methods, 47 participants reported results. One false negative result was reported.

Sample C

The strains of *E. coli* and *K. pneumoniae* were target organisms. Both strains possess the enzyme β -galactosidase and form typical colonies on most MF media at 35/36/37 °C.

In total, 51 participants reported results for MF methods. Two low outliers were reported.

For MPN methods, 46 participants reported results. One low outlier was reported.

General remarks

For MF methods, most participants followed either EN ISO 9308-1:2014 using the enzyme-based chromogenic medium CCA, or Nordic national standards using LES based on lactose fermentation. Despite LES being more selective than CCA, in this PT round the results appear to be somewhat higher with LES.

For MPN methods, most participants (94 %) used ColilertTM-18 (ISO 9308-2:2012). Of these, 80 % used trays with 97 wells and 18 % used trays with 51 wells. As with CCA, ColilertTM is based on the activity of β -D-galactosidase. β -D-galactosidase cleaves ortho-nitrophenol galactoside (ONPG) and change the coloration of the wells to yellow.

The group Other/Unknown in the table 3 used a multiple tube method based on lactose fermentation for the MPN method.

Table 2. Results from analysis of coliform bacteria with MF methods.

Method	Sample A						Sample B						Sample C								
	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>
All results	50	49	202	17	0	0	1	50	47	42	14	0	0	3	51	49	319	10	0	2	0
ISO 9308-1:2014	22	22	181	14	0	0	0	22	20	37	12	0	0	2	23	22	302	10	0	1	0
SFS 3016	12	12	217	24	0	0	0	12	12	40	11	0	0	0	12	11	335	10	0	1	0
SS 028167	11	11	238	18	0	0	0	11	11	50	11	0	0	0	11	11	332	7	0	0	0
"ISO 9308-1:1990"	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Other/Unknown	2	1	-	-	0	0	1	2	1	-	-	0	0	1	2	2	-	-	0	0	0
NS 4788	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Medium																					
m-Endo Agar LES	27	27	225	20	0	0	0	27	27	44	12	0	0	0	27	26	336	9	0	1	0
Chromocult Coliform Agar	22	22	181	14	0	0	0	22	20	37	12	0	0	2	23	22	302	10	0	1	0
Other/Unknown	1	0	-	-	0	0	1	1	0	-	-	0	0	1	1	1	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scale

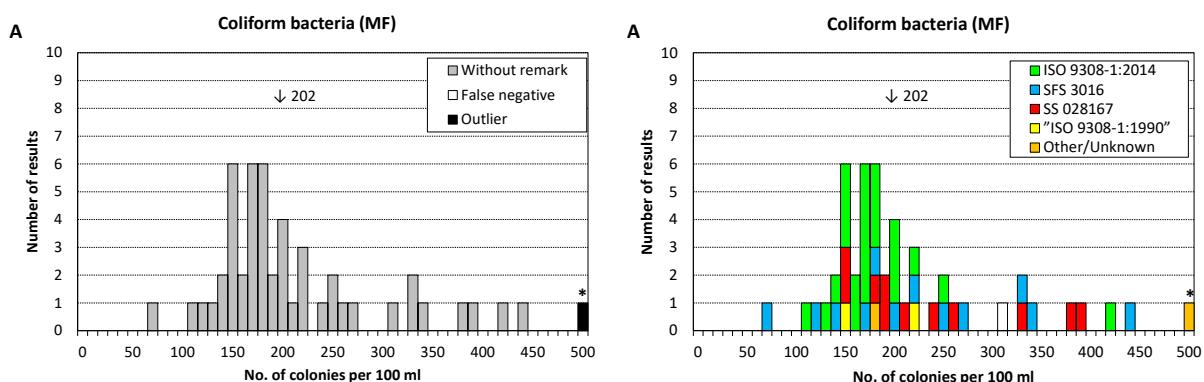
For individual methods, m_{PT} = median value in cfu 100 ml⁻¹

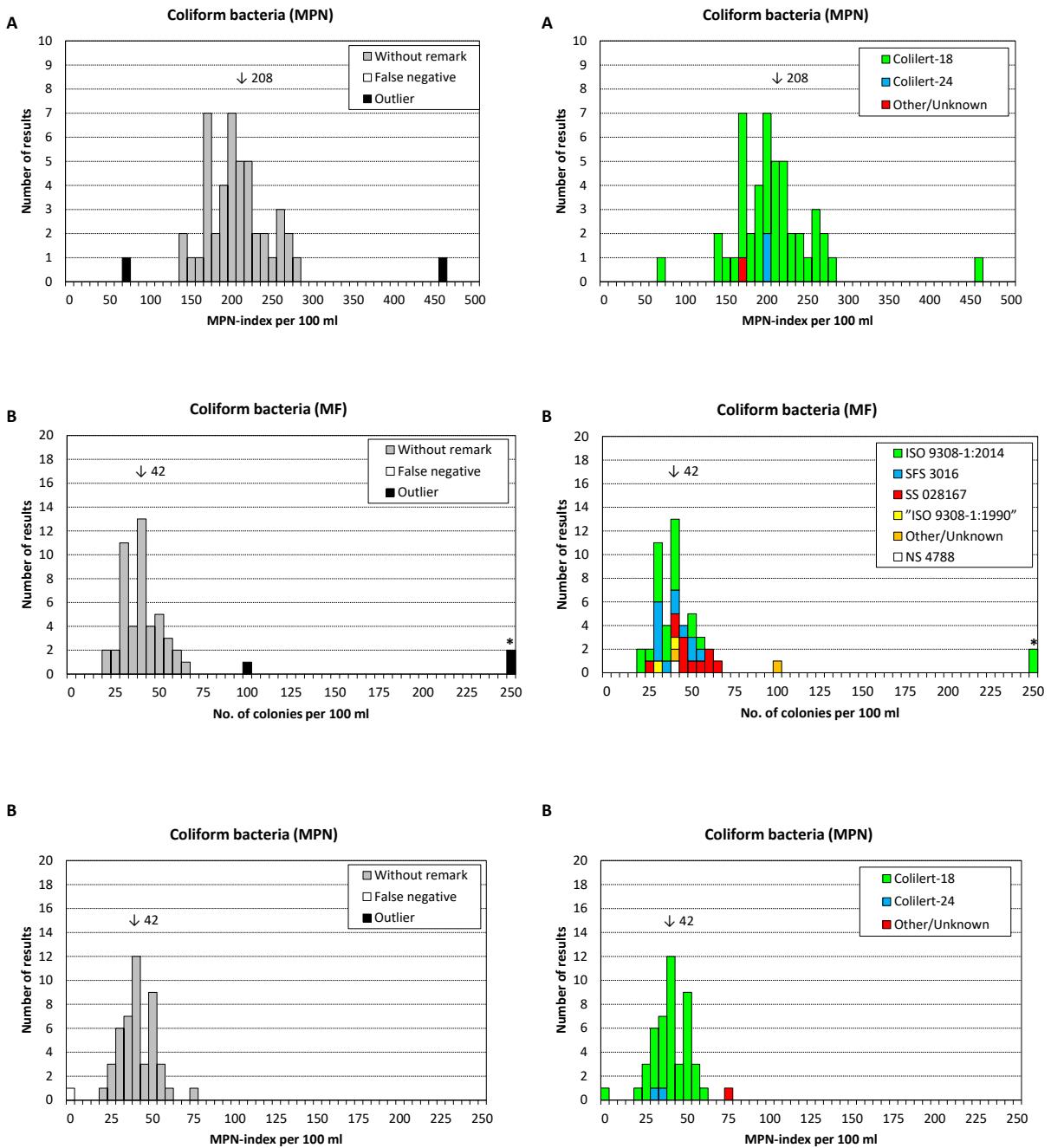
Table 3. Results from analysis of coliform bacteria with MPN methods.

Medium	Sample A						Sample B						Sample C								
	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>
All results	47	45	208	10	0	1	1	47	46	42	12	1	0	0	46	45	344	10	0	1	0
Colilert-18	44	42	209	9	0	1	1	44	43	42	12	1	0	0	43	42	345	9	0	1	0
Colilert-24	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Other/Unknown	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in MPN 100 ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in MPN 100 ml⁻¹





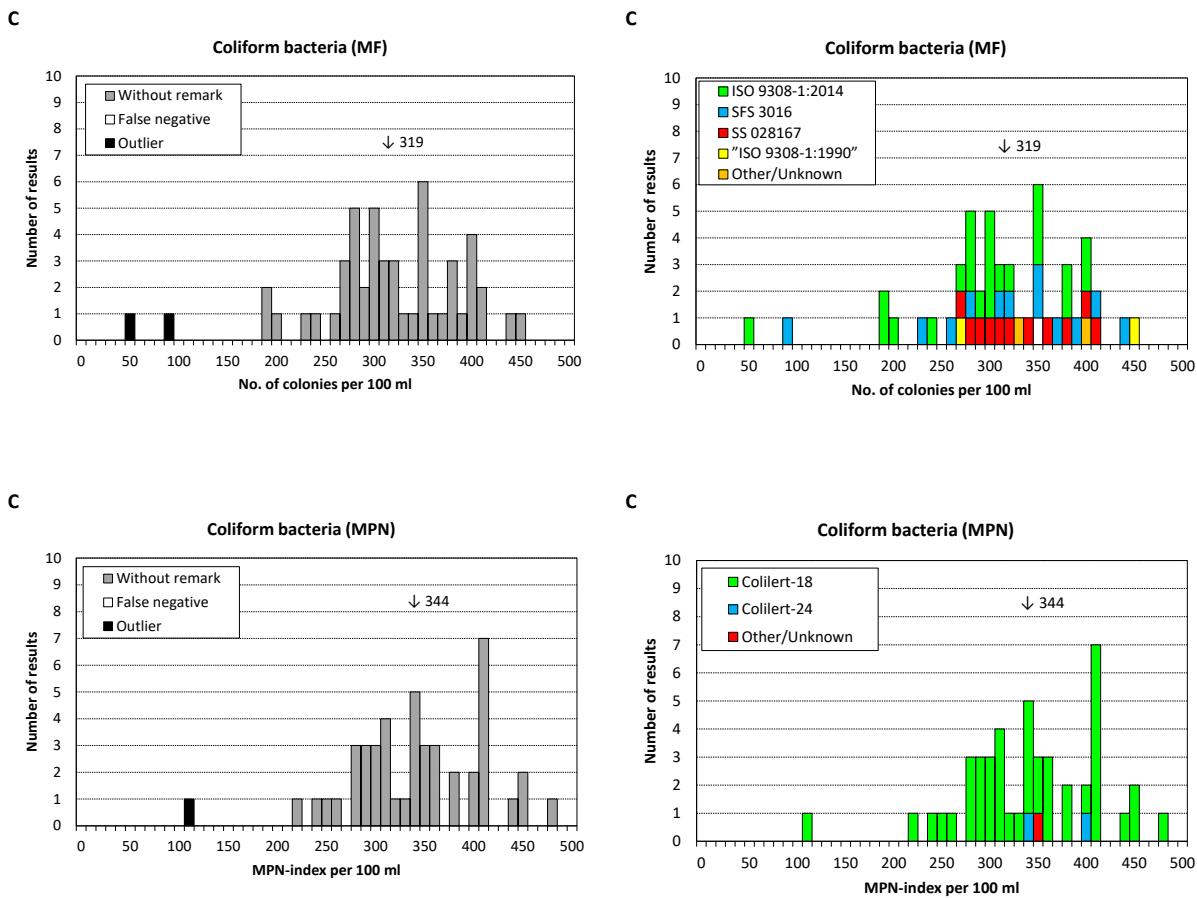


Figure 1. Results from analysis of coliform bacteria

Escherichia coli

Sample A

The strain of *E. coli* was target organism. It forms typical colonies with a metallic sheen on LES and blue colonies on CCA. It possesses the enzyme β -glucuronidase and is detected as *E. coli* with ColilertTM/ColilertTM-18. The strain is positive for indole production and it produces gas in Lactose-Tryptone-Lauryl Sulphate Broth (LTLSB).

In total, 51 participants reported results for MF methods. Three high outliers were reported.

For MPN methods, 47 participants reported results. One high and one low outlier were reported.

Sample B

No target organism was present in the sample. The strain of *K. oxytoca* was however false positive for the analysis. It is able to grow in broth at 44 °C and is indole positive. Depending on the definition of *E. coli* of the analytical method, positive results could be considered accepted.

In total, 51 participants reported results for MF methods. Two false positive results were reported, both following ISO 9308-1:2014.

For MPN methods, 47 participants reported results.

Sample C

The strain of *E. coli* (not identical to that in sample A) was target organism. It has a typical appearance on most MF media, as well as with MPN methods at 35/36/37 °C. The strain is positive for indole production and β -glucuronidase activity, and it produces gas in LTLSB.

In total, 51 participants reported results for MF methods. One high and one low outlier were reported.

For MPN methods, 47 participants reported results. One high and one low outlier were reported.

General remarks

Most participants followed EN ISO 9308-1:2014, Nordic national standards (see table 4) and/or ISO 9308-2:2012. EN ISO 9308 defines *E. coli* as a member of the Enterobacteriaceae that expresses both β -D-galactosidase and β -D-glucuronidase enzymes. On CCA, β -D-galactosidase and β -D-glucuronidase positive reaction appear as dark-blue to violet colonies. On Colilert, yellow wells that also exhibit any degree of fluorescence are regarded as positive for *E. coli*. No further confirmation is needed.

When colonies are isolated from LES or m-FC, confirmation is required. Depending on the method, tests for indole production and/or β -glucuronidase activity are usually performed from oxidase-negative presumptive colonies. In general, participants appear to have performed a confirmation when required so by the method.

The primary MF growth media CCA and LES are incubated at 35/36/37 °C and m-FC at 44/44.5 °C.

Table 4. Results from analysis of *Escherichia coli* with MF methods.

Method	Sample A						Sample B						Sample C								
	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>
All results	51	48	183	14	0	0	3	51	49	-	-	2	-	-	51	49	129	14	0	1	1
ISO 9308-1:2014	25	25	168	10	0	0	0	25	23	-	-	2	-	-	25	24	126	10	0	1	0
SS 028167	11	9	220	11	0	0	2	11	11	-	-	0	-	-	11	11	139	12	0	0	0
SFS 3016 (4088)	11	10	178	20	0	0	1	11	11	-	-	0	-	-	11	11	125	21	0	0	0
"ISO 9308-1:1990"	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	1	-	-	0	0	1
NS 4792	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0
Other/Unknown	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scale

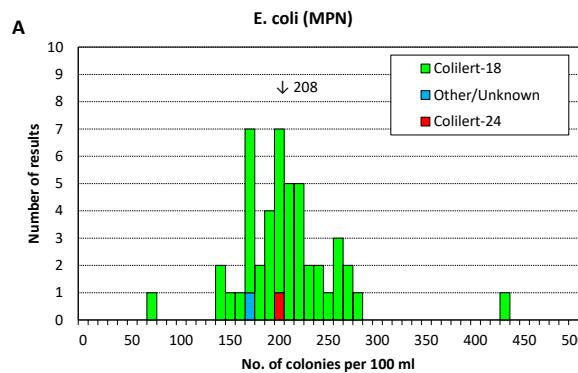
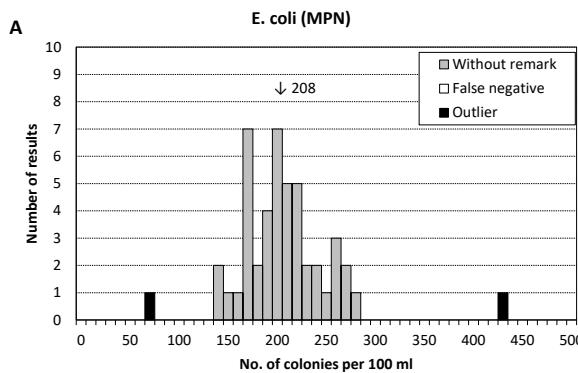
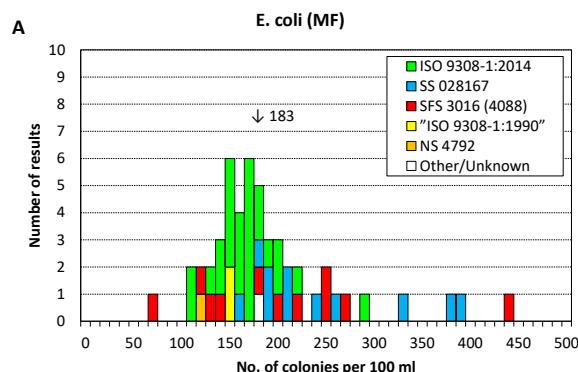
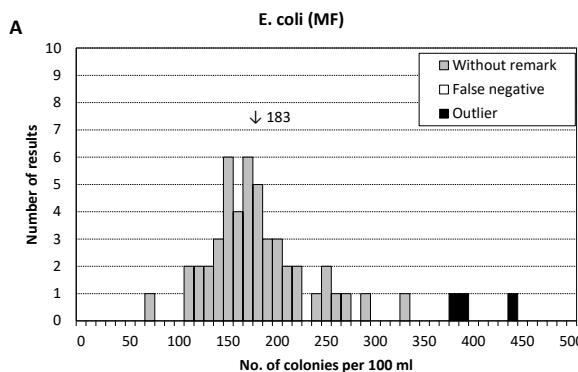
For individual methods, m_{PT} = median value in cfu 100 ml⁻¹

Table 5. Results from analysis of *Escherichia coli* with MPN methods.

Medium	Sample A						Sample B						Sample C								
	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>
All results	47	45	208	10	0	1	1	47	47	-	-	0	-	-	47	45	149	9	0	1	1
Colilert-18	45	43	208	9	0	1	1	45	45	-	-	0	-	-	45	44	147	9	0	1	0
Other/Unknown	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	0	-	-	0	0	1
Colilert-24	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in MPN 100 ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in MPN 100 ml⁻¹



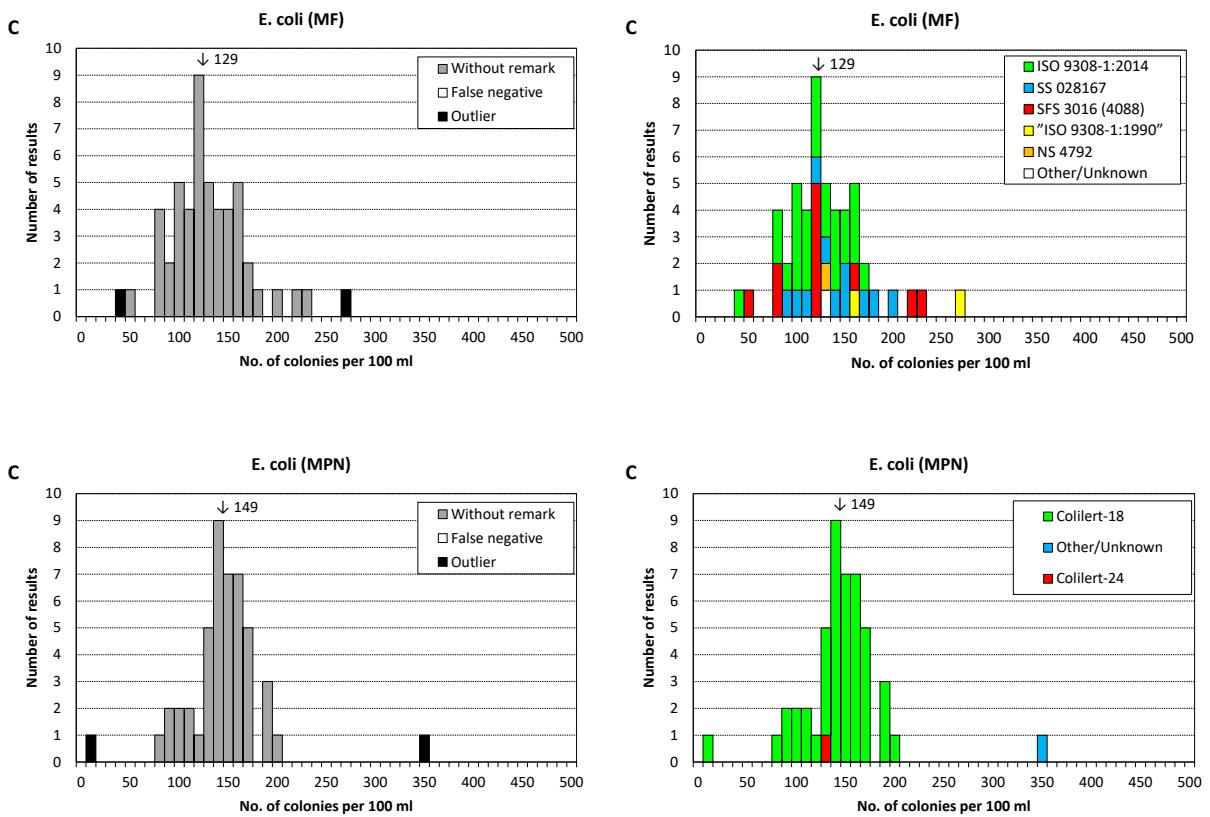


Figure 2. Results from analysis of *Escherichia coli*

Suspected thermotolerant coliform bacteria

Sample A

The strain of *E. coli* was target organism. On m-FC Agar, it forms typical blue colonies at 44/44.5 °C.

In total, 25 participants reported results.

Sample B

No target organism was present in the sample. The strain of *K. oxytoca* was however false positive for the analysis. It is able to grow in broth at 44 °C.

In total, 25 participants reported results. Five false positive results were reported. All from the group Other/Unknown.

Sample C

The strains of *E. coli* and *K. pneumoniae* were target organisms. On m-FC Agar, both strains form blue colonies at 44/44.5 °C.

In total, 25 participants reported results.

General remarks

The parameter suspected thermotolerant coliform bacteria is not evaluated and the median value for performance evaluation is provided only as an information.

In total, 25 participants reported results. Of these, 19 participants (76 %) incubated on m-FC at 44/44.5 °C. The elevated incubation temperature and the addition of rosolic acid make m-FC selective for thermotolerant coliform bacteria.

In table 6, SS 028167, SFS 4088, NS 4792 all reported the use of m-FC.

Table 6. Results from analysis of suspected thermotolerant coliform bacteria.

Method	Sample A						Sample B						Sample C								
	N	n	Med	CV	F	<	>	N	n	Med	CV	F	<	>	N	n	Med	CV	F	<	>
All results	25	25	156*	-	-	-	-	25	20	-	-	-	-	-	25	25	289*	-	-	-	-
Other/Unknown	8	8	162*	-	-	-	-	8	8	-	-	-	-	-	8	8	270*	-	-	-	-
SS 028167	8	8	170*	-	-	-	-	8	8	-	-	-	-	-	8	8	304*	-	-	-	-
SFS 4088	7	7	140*	-	-	-	-	7	7	-	-	-	-	-	7	7	325*	-	-	-	-
NS 4792	2	2	-	-	-	-	-	2	2	-	-	-	-	-	2	2	-	-	-	-	-

Med= Median value in cfu 100 ml⁻¹

* The samples are not evaluated. The values are shown only as an information to the participants.

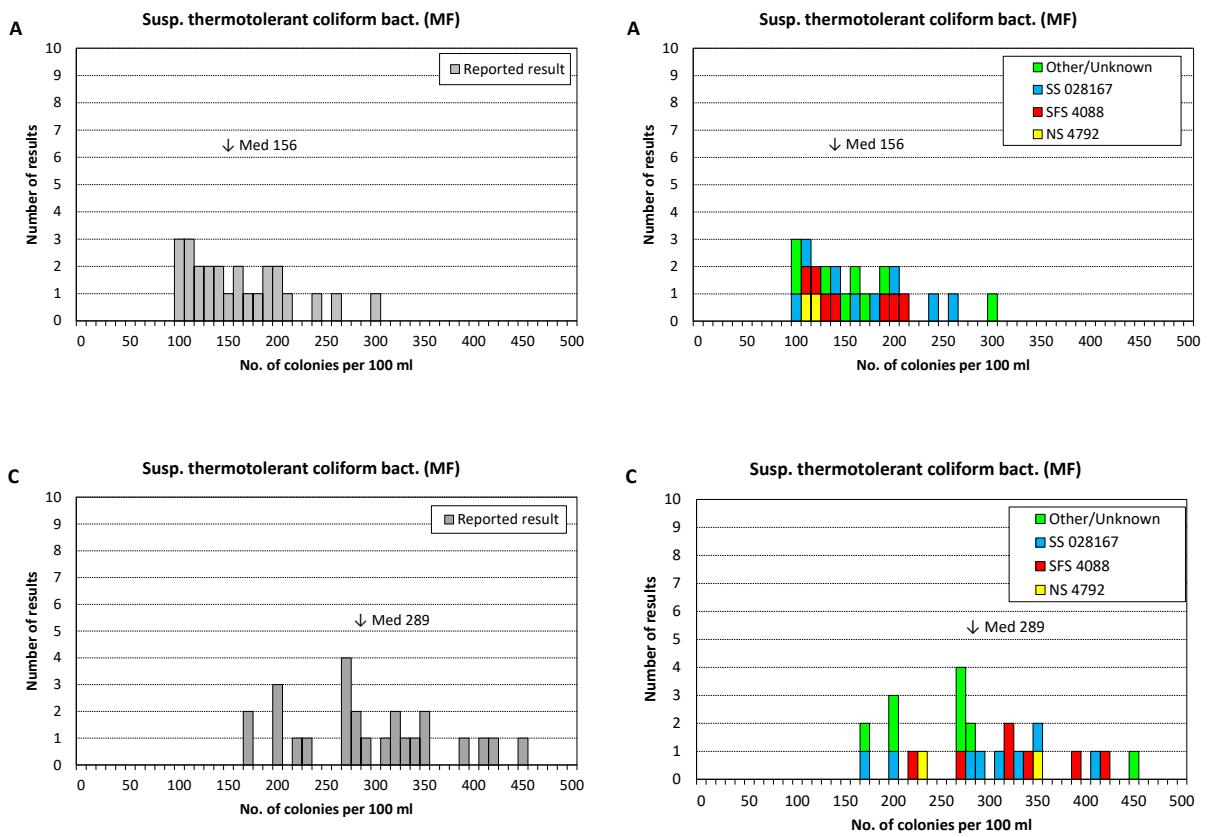


Figure 3. Results from analysis of suspected thermotolerant coliform bacteria

Presumptive and confirmed *Clostridium perfringens*

Sample A

No target organism was present in the sample.

In total, 41 participants reported results for presumptive *C. perfringens*. Two false positive results were reported.

For *C. perfringens*, 26 participants reported results. Three false positive results were reported.

Sample B

The strain of *C. perfringens* was target organism. On Tryptose Sulfite Cycloserine agar (TSC), the colour of the colonies can vary from faint yellow-brown to completely black depending on the condition and reduction potential of the medium.

In total, 41 participants reported results for presumptive *C. perfringens*. Two false negative results and two high outliers were reported.

For *C. perfringens*, 26 participants reported results. Two false negative results and one high outlier were reported.

Sample C

No target organism was present in the sample for *C. perfringens*. However, the strain of *C. bifermentans* was included in the sample as presumptive *C. perfringens*. On TSC, it forms small faint yellow-brown to black colonies. The strain does not possess the enzyme acid phosphatase and should not be reported as confirmed *C. perfringens*.

In total, 41 participants reported results for presumptive *C. perfringens*. Two false negative results as well as one high and one low outlier were reported.

For *C. perfringens*, 26 participants reported results. Ten false positive results were reported, indicating either that no confirmation was made or that the results of the confirmation were misinterpreted.

General remarks

The parameter *C. perfringens* includes spores and vegetative cells of *C. perfringens*. In Sweden, laboratories may report presumptive or confirmed *C. perfringens*.

Most participants (78 %) followed (EN) ISO 14189. According to the standard, presumptive *C. perfringens* is defined as bacteria that produce all shades of black or grey to yellow brown colonies on TSC after anaerobic incubation. *C. perfringens* is presumptive *C. perfringens* that possess the enzyme acid phosphatase.

Six participants reported following ISO/CD 6461-2:2002, based on TSC including four different confirmation tests. One participant reported the use of m-CP Agar incubated at 44 °C. That method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*.

Table 7. Results from analysis of presumptive *C. perfringens*

Method	Sample A						Sample B						Sample C								
	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>
All results	41	39	-	-	2	-	-	41	37	45	16	2	0	2	41	37	598	20	2	1	1
(EN) ISO 14189	32	30	-	-	2	-	-	32	28	42	15	2	0	2	32	28	566	20	2	1	1
ISO/CD 6461-2:2002	6	6	-	-	0	-	-	6	6	46	16	0	0	0	6	6	680	14	0	0	0
Other/Unknown	3	3	-	-	0	-	-	3	3	-	-	0	0	0	3	3	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scale

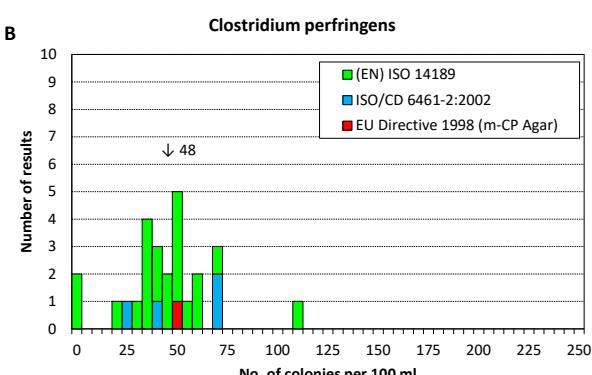
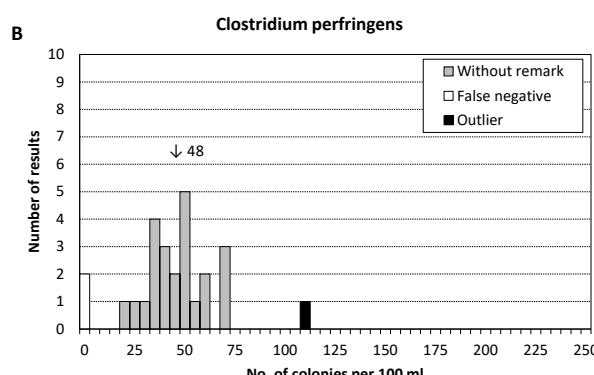
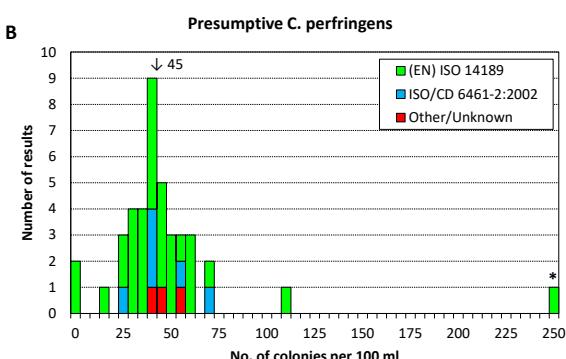
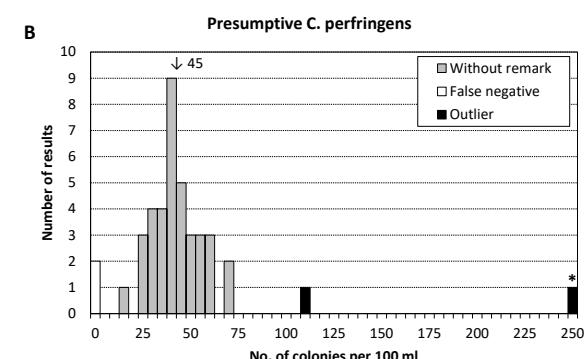
For individual methods, m_{PT} = median value in cfu 100 ml⁻¹

Table 8. Results from analysis of *C. perfringens*

Method	Sample A						Sample B						Sample C								
	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>
All results	26	23	-	-	3	-	-	26	23	48	16	2	0	1	26	16	-	-	10	-	-
(EN) ISO 14189	21	18	-	-	3	-	-	21	18	46	14	2	0	1	21	12	-	-	9	-	-
ISO/CD 6461-2:2002	4	4	-	-	0	-	-	4	4	-	-	0	0	0	4	3	-	-	1	-	-
EU Directive 1998 (m-CP Agar)	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in cfu 100 ml⁻¹



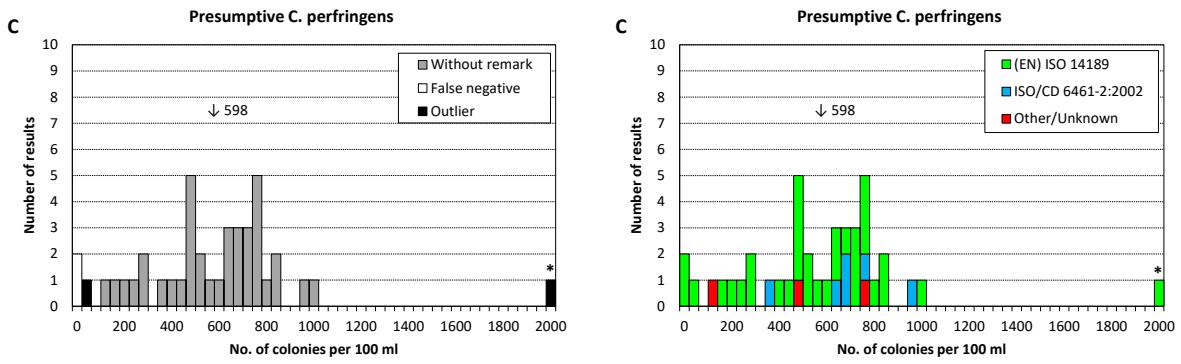


Figure 4. Results from analysis of presumptive and confirmed *Clostridium perfringens*.

Moulds and yeasts

Sample A

The strains of *P. fastigiata* and *C. glabrata* were target organisms for moulds and yeasts, respectively.

In total, 36 participants reported results for moulds. One false negative result and one low outlier were reported.

For yeasts, 36 participants reported results. One false negative result as well as one high and one low outlier were reported.

The false negative results were from the same laboratory and were possibly the result of a mix-up between samples A and B.

Sample B

No target organism was present in the sample.

In total, 35 participants reported results for moulds. Two false positive results were reported.

For yeasts, 35 participants reported results. Three false positive results were reported.

Sample C

No target organism was present in the sample.

In total, 35 participants reported results for moulds. Two false positive results were reported.

For yeasts, 35 participants reported results. One false positive result was reported.

General remarks

Most participants followed the Swedish standard SS 028192. This standard is also partly used in Finland under the national designation SFS 5507. SS 028192 is based on incubation on Rose Bengal agar with chlortetracycline and chloramphenicol at 25 °C for seven days.

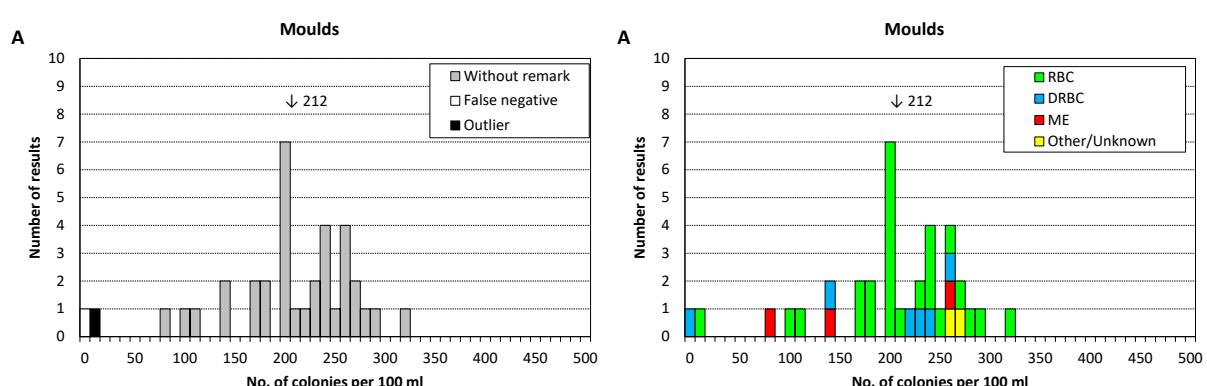
Twenty-five participants used Rose Bengal agar (RBC) including Cooke Rose Bengal agar base, Rose Bengal agar base, Rose Bengal agar, Rose Bengal Chloramphenicol agar. Six participants used DRBC and three participants used Malt Extract agar (ME).

Table 9. Results from analysis of moulds.

Medium	Sample A						Sample B						Sample C					
	N	n	m_{PT}	CV	F	< >	N	n	m_{PT}	CV	F	< >	N	n	m_{PT}	CV	F	< >
All results	36	34	212	13	1	1 0	35	33	-	-	2	- -	35	33	-	-	2	- -
SS 028192	22	22	211	12	0	0 0	22	22	-	-	0	- -	22	22	-	-	0	- -
Other/Unknown	6	5	174	23	0	1 0	5	5	-	-	0	- -	5	4	-	-	1	- -
SFS 5507	5	4	-	-	1	0 0	5	4	-	-	1	- -	5	5	-	-	0	- -
NS (SS) 028192	2	1	-	-	0	1 0	2	1	-	-	1	- -	2	1	-	-	1	- -
NMKL 98:2005 (modified)	1	1	-	-	0	0 0	1	1	-	-	0	- -	1	1	-	-	0	- -
Medium																		
RBC	25	24	212	13	0	1 0	25	25	-	-	0	- -	25	25	-	-	0	- -
DRBC	6	5	216	11	1	0 0	6	5	-	-	1	- -	6	6	-	-	0	- -
ME	3	3	-	-	0	0 0	3	3	-	-	0	- -	3	2	-	-	1	- -
Other/Unknown	2	2	-	-	0	0 0	1	0	-	-	1	- -	1	0	-	-	1	- -

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scaleFor individual methods, m_{PT} = median value in cfu 100 ml⁻¹**Table 10.** Results from analysis of yeasts.

Medium	Sample A						Sample B						Sample C					
	N	n	m_{PT}	CV	F	< >	N	n	m_{PT}	CV	F	< >	N	n	m_{PT}	CV	F	< >
All results	36	33	252	10	1	1 1	35	32	-	-	3	- -	35	34	-	-	1	- -
SS 028192	22	22	254	8	0	0 0	22	21	-	-	1	- -	22	22	-	-	0	- -
Other/Unknown	6	4	-	-	0	1 1	5	4	-	-	1	- -	5	4	-	-	1	- -
SFS 5507	5	4	-	-	1	0 0	5	4	-	-	1	- -	5	5	-	-	0	- -
NS (SS) 028192	2	1	-	-	0	1 0	2	2	-	-	0	- -	2	2	-	-	0	- -
NMKL 98:2005 (modified)	1	1	-	-	0	0 0	1	1	-	-	0	- -	1	1	-	-	0	- -
Medium																		
RBC	25	24	253	9	0	1 0	25	24	-	-	1	- -	25	25	-	-	0	- -
DRBC	6	5	269	7	1	0 0	6	5	-	-	1	- -	6	6	-	-	0	- -
ME	3	2	-	-	0	0 1	3	2	-	-	1	- -	3	2	-	-	1	- -
Other/Unknown	2	2	-	-	0	0 0	1	1	-	-	0	- -	1	1	-	-	0	- -

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scaleFor individual methods, m_{PT} = median value in cfu 100 ml⁻¹

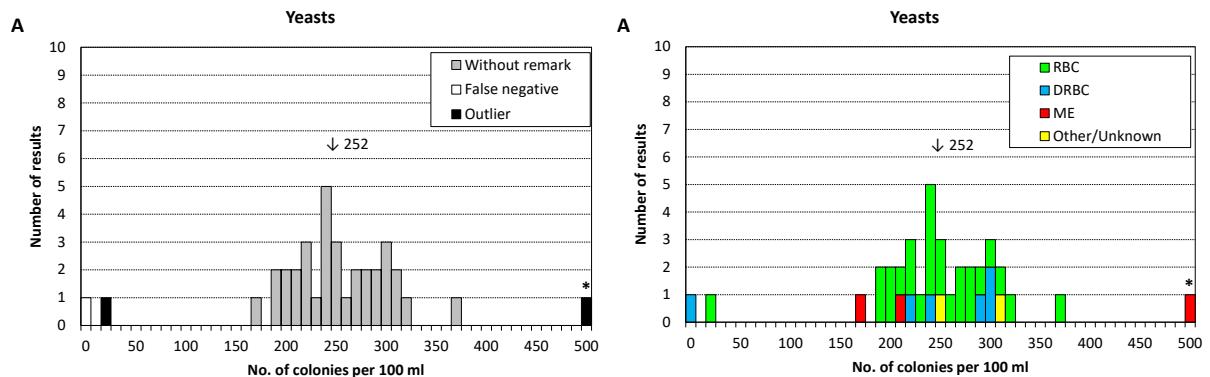


Figure 5. Results from analysis of moulds and yeasts.

Actinomycetes

Sample A

No target organism was present in the sample.

In total, 25 participants reported results.

Sample B

An actinomycete within the group *Streptomyces* sp. was target organism.

In total, 25 participants reported results. One high outlier was reported.

Sample C

An actinomycete within the group *Streptomyces* sp. was target organism (identical to that in sample B).

In total, 25 participants reported results. Three false negative were reported.

General remarks

In Sweden, actinomycetes is included as a parameter for drinking water monitoring according to Swedish regulations (LIVSFS 2022:12). The parameter is analysed according to the Swedish standard for actinomycetes in water, SS 028212 (1994), using Actinomycete Isolation Agar (ACTA) including cycloheximide. Three participants used other methods, with natamycin as the selective substance.

Note: One result was excluded from statistical evaluation in sample C after discussion with the participant.

Table 11. Results from analysis of *Actinomycetes*

Method	Sample A						Sample B						Sample C								
	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>	N	n	m_{PT}	CV	F	<	>
All results	25	25	-	-	0	-	-	25	24	30	17	0	0	1	24	21	29	16	3	0	0
SS 028212	21	21	-	-	0	-	-	21	20	31	15	0	0	1	20	18	29	17	2	0	0
Other/Unknown	3	3	-	-	0	-	-	3	3	-	-	0	0	0	3	2	-	-	1	0	0

For "All results", m_{PT} = assigned value, robust mean value in cfu 100 ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in cfu 100 ml⁻¹

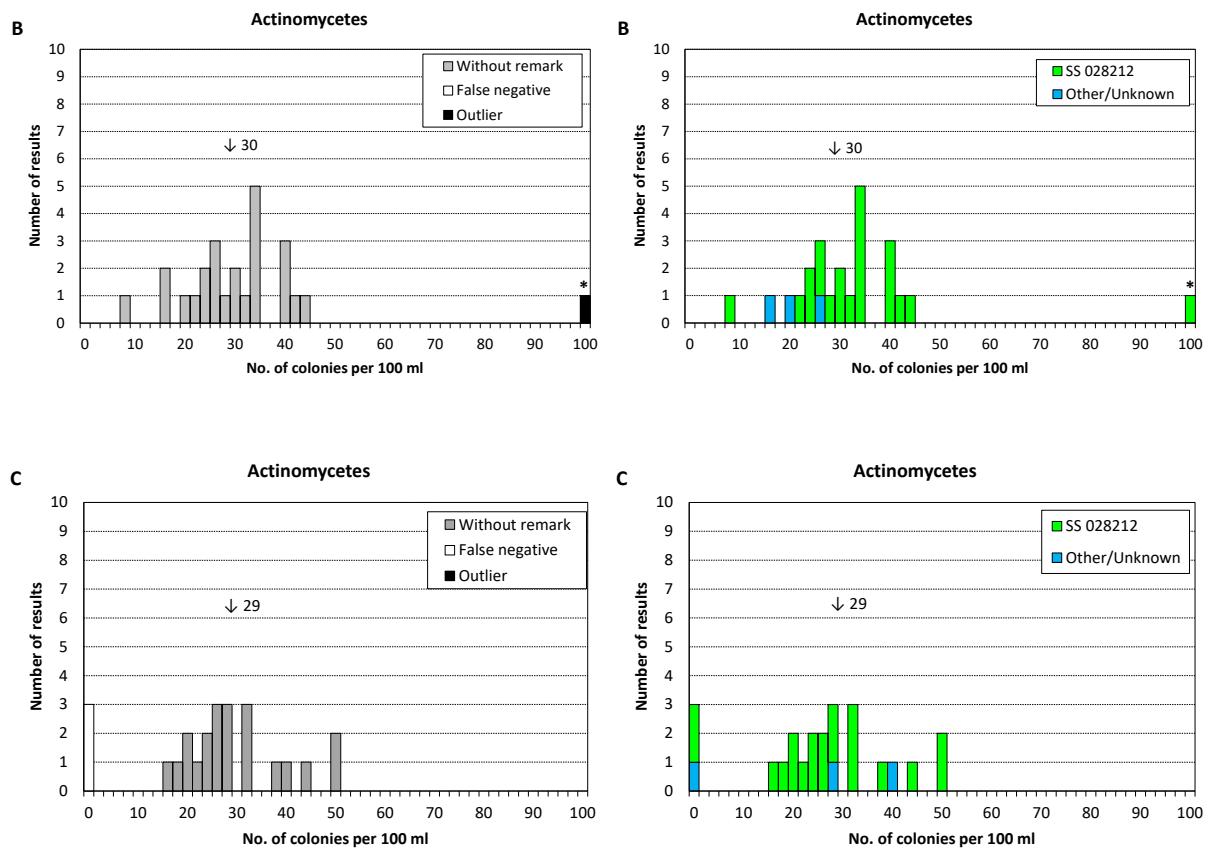


Figure 6. Results from analysis of actinomycetes.

Culturable microorganisms, 72 hours incubation at 22 °C

Sample A

The strain of *S. maltophilia* was the main target organism.

In total, 70 participants reported results. One low and one high outlier were reported.

Sample B

The strain of *S. saprophyticus* was the main target organism.

In total, 70 participants reported results. One low outlier was reported.

Sample C

The strain of *M. caseolyticus* was the main target organism.

In total, 70 participants reported results. Two low outliers were reported.

General remarks

Sixty-seven out of 69 participants followed EN ISO 6222:1999, which is based on a pour-plate method with Yeast extract Agar (YEA). Eight participants reported the use of Plate Count Agar (PCA) instead of YEA. One participant followed Standard Methods, 9215 B [2] and in the group Other/Unknown 3MTM PetrifilmTM Aerobic Count Plate was used.

Fifty-four participants reported to include both mould and yeasts in the result. Four participants included only yeasts and 11 participants did not include either mould or yeast.

Note: One participant incubated for 48 hours at 35 °C; the results are excluded from statistical evaluation.

Table 12. Results from analysis of culturable microorganisms, 72 hours incubation at 22±2 °C.

Method	Sample A							Sample B							Sample C						
	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>
All results	69	67	55	12	0	1	1	69	68	54	12	0	1	0	69	67	31	10	0	2	0
ISO 6222:1999	67	65	55	11	0	1	1	67	66	56	9	0	1	0	67	65	32	10	0	2	0
Standard Methods, 9215	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Other/Unknown	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Medium																					
Yeast extract Agar	58	57	55	12	0	0	1	58	58	57	9	0	0	0	58	57	32	9	0	1	0
Plate Count Agar	8	7	49	7	0	1	0	8	8	52	11	0	0	0	8	7	32	13	0	1	0
Other/Unknown	3	3	-	-	0	0	0	3	2	-	-	0	1	0	3	3	-	-	0	0	0

Magnification	5–11,9x	28	28	58	11	0	0	0	28	28	57	8	0	0	0	28	28	33	10	0	0	0
5–11,9x	28	28	58	11	0	0	0	0	28	28	57	8	0	0	0	28	28	33	10	0	0	0
1.1–4.9x	22	20	54	13	0	1	1	1	22	22	55	12	0	0	0	22	21	31	10	0	1	0
None	19	19	50	7	0	0	0	0	19	18	57	7	0	1	0	19	18	31	7	0	1	0

For "All results", m_{PT} = assigned value, robust mean value in cfu ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in cfu ml⁻¹

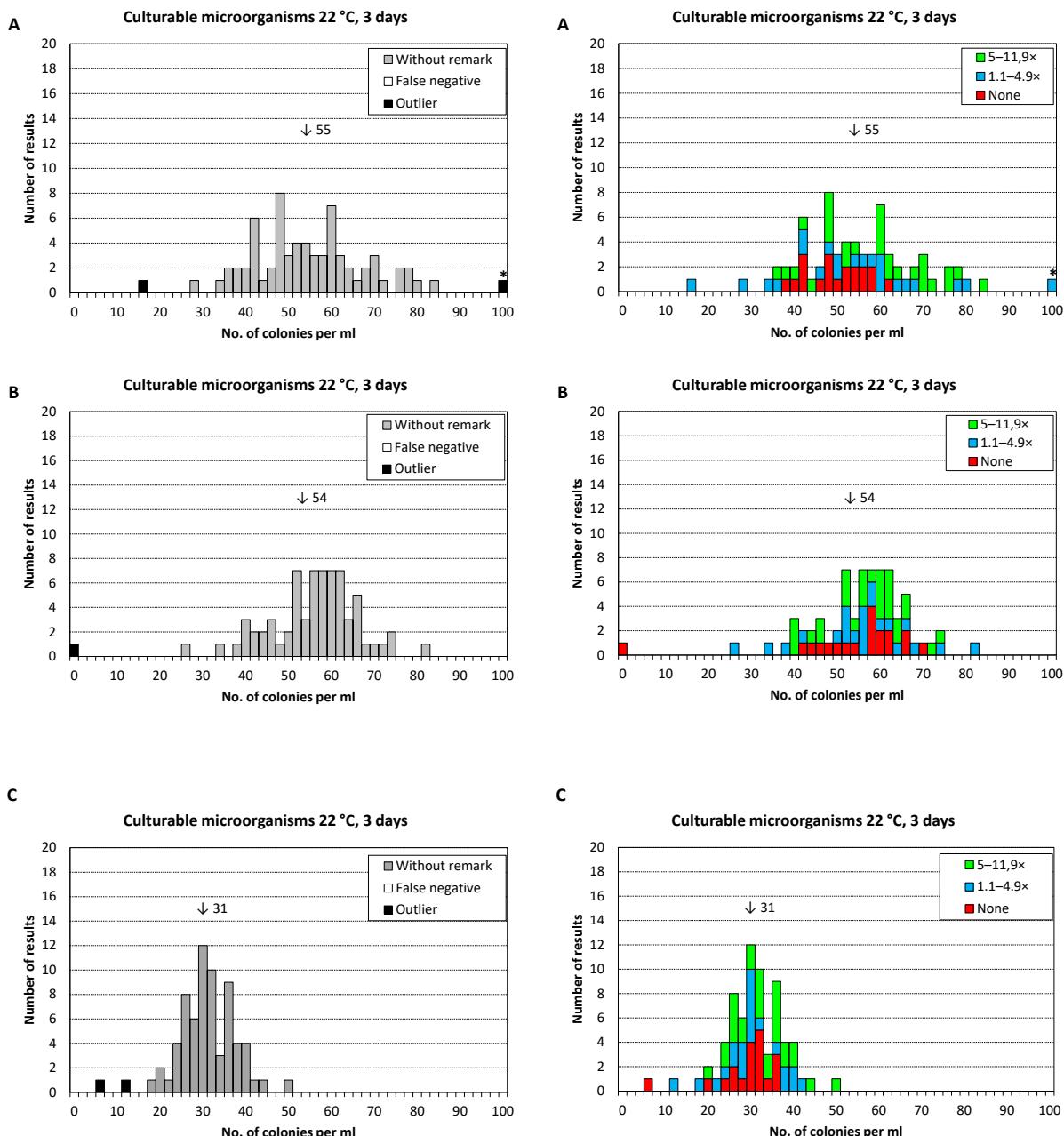


Figure 8. Results from analysis of culturable microorganisms, 72 hours incubation at 22 °C

Slow-growing bacteria 22 °C, 7 days

Sample A

No specific slow-growing bacterium was included in the sample, but the strain of *S. maltophilia* as well as other bacteria included in the sample will form colonies on YEA and Reasoner's 2 Agar (R2A) at 22 °C after 7 days.

In total, 41 participants reported results.

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

Sample B

No specific slow-growing bacterium was included in the sample, but the strain of *S. saprophyticus* and individual colonies of the coliform bacteria and the actinomycete may form colonies on YEA and R2A at 22 °C after 7 days.

In total, 41 participants reported results.

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

Sample C

The strain of *Sphingomonas* sp. was the main target organism.

In total, 41 participants reported results. Two low outliers were reported.

General remarks

In Sweden, slow-growing bacteria is included as a parameter for drinking water monitoring according to Swedish regulations (LIVSFS 2022:12). Before 2003 a Swedish standard was available, but the parameter is currently analysed according to a modified version of EN ISO 6222:1999. The modifications are prolonged incubation time (seven days) and that only bacteria should be included in the result. The standard method is also specified with incubation at 22 ± 1 °C and that magnification should be used when reading the plates (at least 4×, preferentially 10×). There is an ongoing process within ISO to develop a standard method for the parameter "slow-growing microorganisms". The current proposal is to use the low nutrient media R2A instead of YEA and to use a spread-plate technique instead of a pour-plate technique.

Samples A and B did not include any specific slow-growing bacteria and are not included in the evaluation. This is due to insufficient data to guarantee sample homogeneity for the parameter slow-growing bacteria. Therefore, the median value for performance evaluation is provided only as an information.

Sample C included a slow-growing *Sphingomonas* sp. bacterium. It often forms small colonies, and magnification is usually needed. As shown in table 9, the median value increases with the magnification.

Most participants used YEA and six participants used R2A. Twenty-four (59 %) participants reported that they included only bacteria in the result, five (12 %) participants only included yeasts and 12 (29 %) participants included both moulds and yeasts.

Table 13. Results from analysis of slow-growing bacteria 22 °C, 7 days

Method	Sample A						Sample B						Sample C						
	N	n	Med	CV	F	< >	N	n	Med	CV	F	< >	N	n	m_{PT}	CV	F	< >	
All results	41	41	61*	-	-	-	41	41	63*	-	-	-	41	39	463	21	0	2	0
ISO 6222:1999 mod.	37	37	61*	-	-	-	37	37	63*	-	-	-	37	35	461	23	0	2	0
Standard Methods, 9215	3	3	-	-	-	-	3	3	-	-	-	-	3	3	-	-	0	0	0
Other/Unknown	1	1	-	-	-	-	1	1	-	-	-	-	1	1	-	-	0	0	0
Medium																			
Yeast extract Agar	27	27	61*	-	-	-	27	27	63	-	-	-	27	27	475	24	0	0	0
Other/Unknown	8	8	59*	-	-	-	8	8	63	-	-	-	8	6	387	25	0	2	0
R2A medium	6	6	65*	-	-	-	6	6	66	-	-	-	6	6	506	8	0	0	0
Magnification																			
≥5×	26	26	61*	-	-	-	26	26	64*	-	-	-	26	25	508	22	0	1	0
1.1–4.9×	8	8	55*	-	-	-	8	8	59*	-	-	-	8	8	422	20	0	0	0
None	4	4	-	-	-	-	4	4	-	-	-	-	4	3	341**	-	0	1	0
Other/Unknown	3	3	-	-	-	-	3	3	-	-	-	-	3	3	-	-	0	0	0

For "All results", m_{PT} = assigned value, robust mean value in cfu ml⁻¹, re-transformed to the cfu scale

For individual methods, m_{PT} = median value in cfu ml⁻¹

* The samples are not evaluated. The values are shown only as an information to the participants.

** Median value in cfu ml⁻¹ value is shown for comparison despite few results

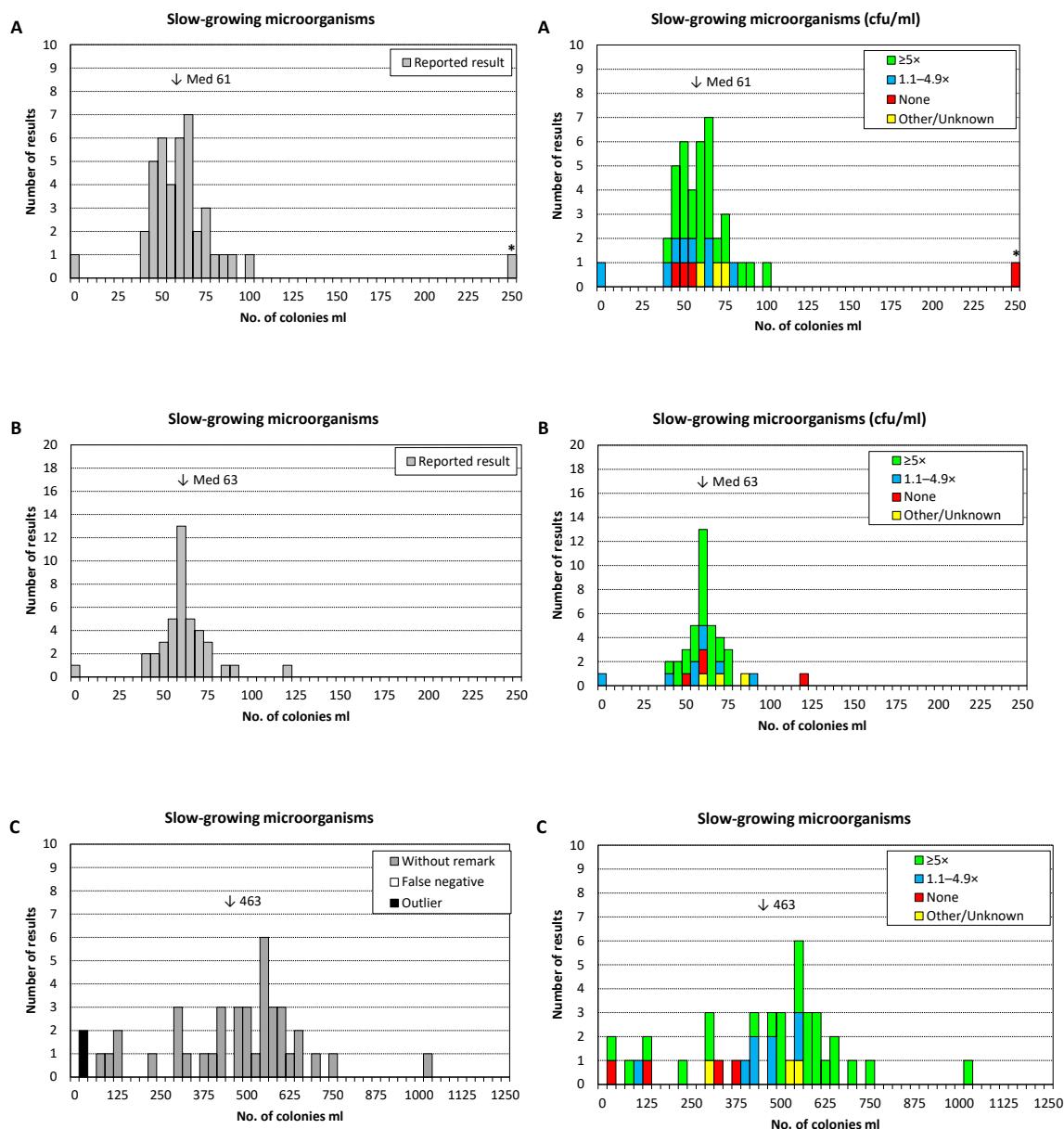


Figure 9. Results from analysis of slow-growing bacteria

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 [3].

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

Box plots and numbers of deviating results for each participant

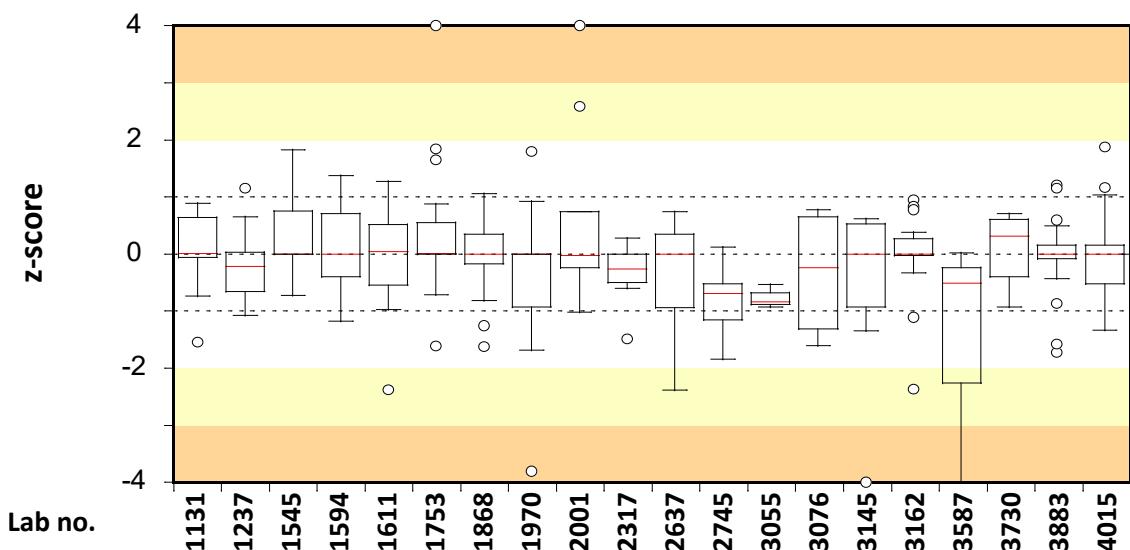
Box plots are based on the z -scores listed in Annex 2, and give a comprehensive view of the performance of each participant. The range of z -scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred around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

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

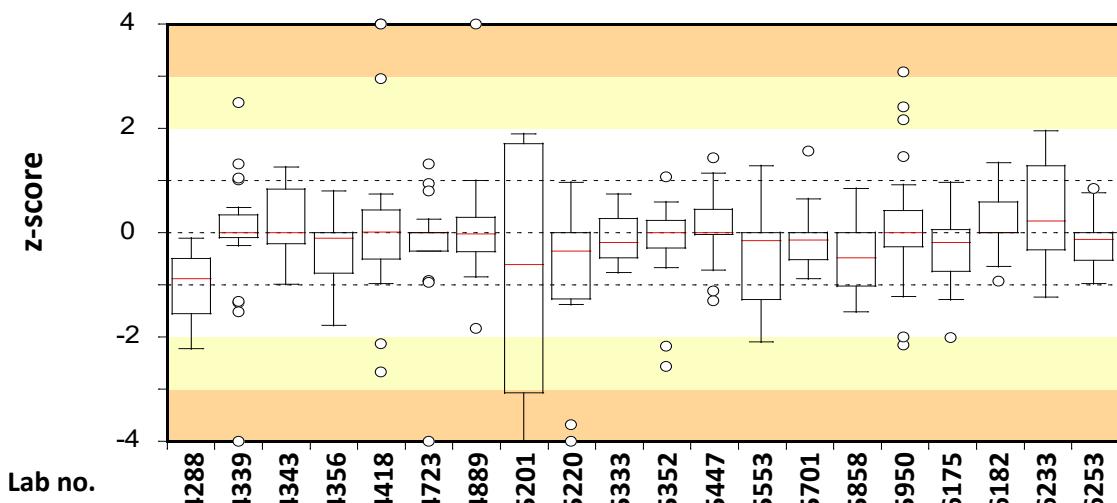
The participant's median value is illustrated by a horizontal line in the box. Each box includes 50 % of a participant's results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box. A circle is for technical reasons shown in the plot when a value deviates to certain degree * from the other values. This does not by itself indicate that the value is an outlier.

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

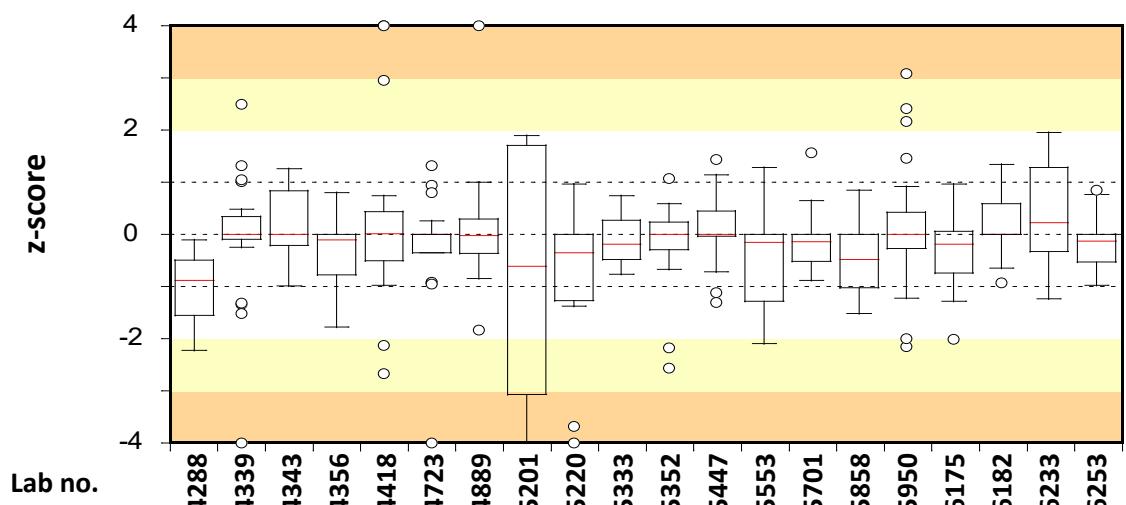
* $< [\text{lowest value in the box} - 1.5 \times (\text{highest value in the box} - \text{lowest value in the box})]$
or
 $> [\text{highest value in the box} + 1.5 \times (\text{highest value in the box} - \text{lowest value in the box})]$.



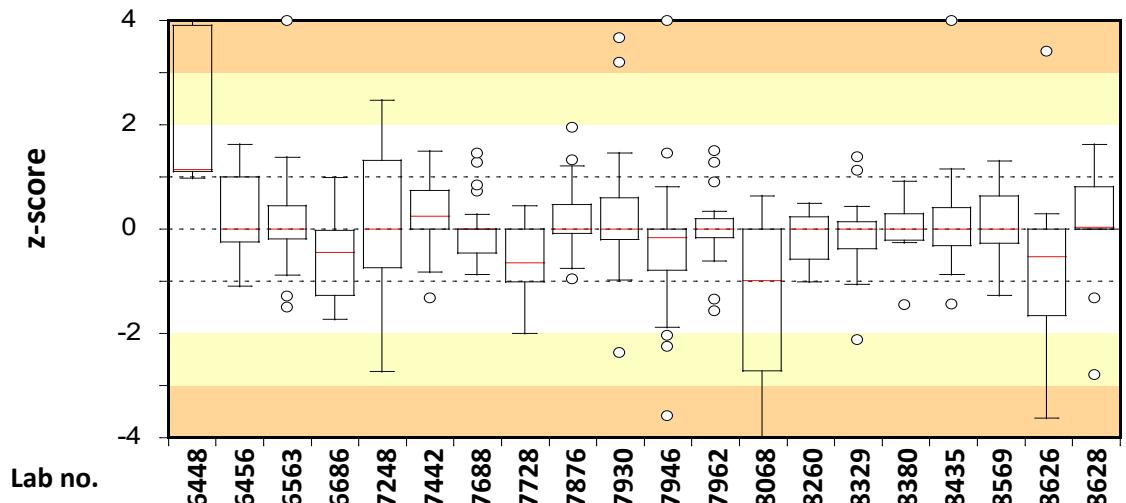
	1131	1237	1545	1594	1611	1753	1868	1970	2001	2317	2637	2745	3055	3076	3145	3162	3587	3730	3883	4015
No. of results	15	24	36	18	21	24	33	24	12	15	15	15	3	6	9	24	3	9	30	24
False positive	0	2	0	0	0	0	1	1	1	0	1	1	2	0	0	0	0	0	1	0
False negative	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
High outliers	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0



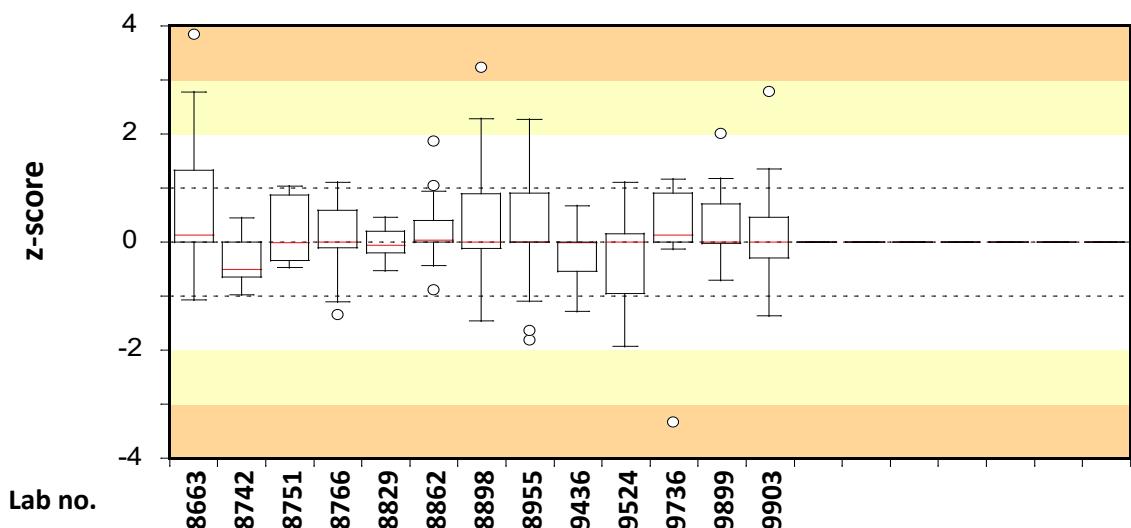
	4288	4339	4343	4356	4418	4723	4889	5201	5220	5333	5352	5447	5553	5701	5858	5950	6175	6182	6233	6253
No. of results	3	28	22	21	28	21	18	7	22	3	22	25	9	9	12	31	8	22	15	9
False positive	0	0	0	0	6	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
False negative	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0
Low outliers	0	1	0	0	0	2	0	2	3	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0



	No. of results	False positive	False negative	Low outliers	High outliers
No. of results	3	28	22	21	28
False positive	0	0	0	0	6
False negative	0	1	0	0	2
Low outliers	0	1	0	0	0
High outliers	0	0	0	0	0



	No. of results	False positive	False negative	Low outliers	High outliers
No. of results	5	15	28	12	28
False positive	0	0	0	0	0
False negative	0	0	0	0	1
Low outliers	0	0	0	0	0
High outliers	2	0	2	0	0



No. of results	24	6	12	33	12	33	30	36	33	12	24	30	24
False positive	0	0	0	0	0	0	0	0	0	0	0	0	1
False negative	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	1	0	0
High outliers	1	0	0	0	0	0	1	0	0	0	0	0	0

Test material and quality control

Test material

Each participant received three samples with freeze-dried microorganisms, designated 1–3. The test material was freeze-dried in 0.5 ml portions in glass vials, as described by Peterz and Steneryd [4]. Before analysing the samples, the contents of each vial should be reconstituted in 800 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.

Sample	Microorganism	Strain			
		SLV no. ¹	Origin	Reference ²	cfu/100 ml ³
A	<i>Escherichia coli</i>	SLV-165	Drinking water	CCUG 43600	210
	<i>Aeromonas caviae</i>	SLV-533	Water	CCUG 48892	140
	<i>Candida glabrata</i>	SLV-052	-	CBS 275 2005	265
	<i>Phialophora fastigiata</i>	SLV-504	Water	-	220
	<i>Stenotrophomonas maltophilia</i>	SLV-041	-	CCUG 46537	60*
B	<i>Klebsiella oxytoca</i>	SLV-553	Water	-	30
	<i>Citrobacter freundii</i>	SLV-091	Water	CCUG 43597	30
	<i>Clostridium perfringens</i>	SLV-442	-	CCUG 43593	50
	<i>Streptomyces species</i>	SLV-548	Reservoir water	-	30
	<i>Staphylococcus saprophyticus</i>	SLV-013	-	CCUG 45100	50*
C	<i>Escherichia coli</i>	SLV-084	Drinking water	-	150
	<i>Klebsiella pneumoniae</i>	SLV-186	Vegetarian kebab	CCUG 45102	190
	<i>Clostridium bifermentans</i>	SLV-009	Fish	CCUG 43592	210
	<i>Sphingomonas species</i>	SLV-547	Water	-	670*
	<i>Streptomyces species</i>	SLV-548	Reservoir water	-	35
	<i>Macrococcus caseolyticus</i>	SLV-462	-	CCUG 35411	25*

¹ Internal strain identification no. at the Swedish Food Agency

² Culture collection: CBS: Centraalbureau voor Schimmelcultures (Westerdijk Institute), CCUG: Culture Collection University of Gothenburg

³ cfu = colony forming units

* indicates cfu per ml

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 the sample mixture is previously approved for homogeneity, but 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. (For definitions of I_2 , and T , see references [5] and [6] respectively.)

Table 15. Concentration mean (m), I_2 and T values from the quality control of the sample; m is expressed in cfu (colony forming units) per 100 ml of sample for MF methods and per 1 ml for pour plate methods.

Analysis and method	Sample A ¹			Sample B ¹			Sample C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Coliform bacteria (MF) SS-EN ISO 9308-1:2014	36 ³	0.72	1.33	53	1.36	1.35	34 ³	0.30	1.21
Suspected thermotolerant colif. bact. (MF) SS 028167	15 ³	0.57	1.45	-	-	-	32 ³	1.32	1.51
<i>Escherichia coli</i> (MF) SS-EN ISO 9308-1:2014	21 ³	1.33	1.59	-	-	-	15 ³	0.19	1,25
Presumptive <i>Clostridium perfringens</i> (MF) SS-EN ISO 14189:2016	-	-	-	51	1.21	1.37	21 ³	0.90	1.50
Moulds (MF) SS 028192	22 ³	0.49	1.34	-	-	-	-	-	-
Yeasts (MF) SS 02819	26 ³	1.00	1.47	-	-	-	-	-	-
Actinomycetes (MF) SS 028212	-	-	-	31	1.32	1.48	3 ³	0.78	2.46
Culturable microorg., 72 h 22 °C (pour plate) SS-EN ISO 6222:1999	67	1.53	1.35	48	2.66	1.59	30	0.84	1.39
Slow-growing bacteria, 7d 22 °C (pour plate) SS-EN ISO 6222:1999 modified	-	-	-	-	-	-	60 ²	0.58	1.22

– No target organism or no value

¹n = 5 vials analysed in duplicate

²cfu per 0.1 ml of sample

³cfu per 10 ml of sample

References

1. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Standard Methods for the Examination of Water and Wastewater, www.standardmethods.org
3. Ilbäck J and Blom L. 2023. Protocol – Microbiological Proficiency Testing, Swedish Food Agency.
4. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
5. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockefer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
6. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.

Annex 1. Results of the participating laboratories

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria (MPN)			E. coli (MPN)			Presumptive C. perfringens			Clostridium perfringens			Moulds			Yeasts			Actinomycetes			Culturable microorganisms 22 °C, 3 days (cfu/ml)		
					A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
1131	1 2 3	-	-	-	-	-	-	-	-	-	179	52	345	179	0	166	0	54	280	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54	63	37	
1237	3 1 2	-	-	-	250	30	400	170	<2	170	150	<1	110	170	40	290	170	<1	150	-	-	170	55	170	-	-	-	-	-	-	-	-	48	63	30		
1545	1 3 2	470	49	410	260	49	410	260	0	410	260	0	200	199	35	343	199	0	154	0	70	660	0	70	0	220	0	0	300	0	0	0	35	29	70	64	28
1594	3 2 1	-	-	-	250	51	325	200	0	325	250	0	120	185	58	310	185	0	120	-	-	-	-	-	-	-	-	-	-	-	-	-	58	71	31		
1611	3 2 1	340	52	410	140	52	410	132	0	390	140	0	160	214	35	411	214	0	140	-	-	-	-	-	-	-	-	-	-	-	-	-	56	57	29		
1753	2 1 3	-	-	-	-	-	-	-	-	-	228	52	345	228	0	137	0	25	740	-	-	-	327	0	0	218	0	0	0	200	0	79	57	33			
1868	1 2 3	174	55	273	174	55	273	-	-	-	174	0	137	225	41	292	225	0	118	0	43	267	0	43	267	200	0	0	272	0	0	0	32	32	61	59	35
1970	1 3 2	300	33	450	150	33	450	300	33	450	150	0	160	-	-	-	0	26	690	0	26	0	140	0	0	300	0	0	-	-	-	43	38	12			
2001	3 1 2	-	-	-	420	5300	300	-	-	-	170	<1	134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54	42	36		
2317	3 2 1	170	26	320	170	26	320	-	-	-	164	0	120	-	-	-	0	0	480	0	0	0	480	-	-	-	-	-	-	-	-	-	47	51	33		
2637	1 3 2	-	-	-	-	-	-	-	-	-	228	39	222	228	<1	93	<1	35	640	<1	35	640	-	-	-	-	-	-	-	-	-	-	40	59	36		
2745	3 1 2	320	36	208	130	36	208	130	36	208	130	22	89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	46	32			
3055	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	44	28		
3076	2 3 1	160	13	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	65	22		
3145	3 1 2	-	-	-	-	-	-	-	-	-	172	48	260	172	0	166	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	63	1	32			
3162	2 1 3	-	-	-	-	-	-	-	-	-	219	43	411	219	0	172	0	56	690	-	-	-	100	0	0	200	0	0	0	27	29	60	58	31			
3587	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55	48	6			
3730	1 3 2	800	27	170	-	-	-	240	<1	170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	61	32			
3883	2 1 3	-	-	-	180	57	400	-	-	-	180	<1	130	214	26	352	214	<1	146	-	-	182	0	0	232	0	0	0	43	50	48	52	38				
4015	2 3 1	-	-	-	-	-	-	-	-	-	214	35	308	214	0	131	0	28	527	-	-	-	-	-	-	-	-	-	-	-	-	-	29	53	26		
4288	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
4339	3 1 2	120	46	390	120	46	390	110	0	320	120	0	230	200	54	250	200	0	12	0	49	0	0	49	0	240	0	0	240	0	0	0	60	57	40		
4343	2 1 3	-	-	-	-	-	-	-	-	-	249	36	411	249	0	172	0	32	400	-	-	200	0	0	226	0	0	0	31	26	65	72	37				
4356	1 3 2	340	33	260	227	33	260	140	0	270	227	0	87	142	33	308	142	0	131	0	46	580	0	46	0	-	-	-	-	-	-	-	51	53	31		
4418	3 2 1	200	42	310	200	42	310	100	0	270	190	0	100	170	79	350	170	0	350	160	0	3800	160	0	300	89	0	30	900	23	280	-	-	56	55	36	
4723	1 3 2	-	-	-	180	360	280	-	-	-	180	0	160	140	39	290	140	0	140	0	40	811	-	-	17	0	0	22	0	0	0	31	100	53	53	40	
4889	1 3 2	-	-	-	180	360	280	-	-	-	180	0	160	140	39	290	140	0	140	-	-	0	53	0	-	-	-	-	-	-	-	-	69	61	29		
5201	3 1 2	-	-	-	-	50	-	-	-	-	295	154	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	80	27.3	39.3	
5220	2 3 1	203	37	283	118	35	283	169	11	270	118	0	124	249	30	345	249	0	133	-	-	-	-	-	-	200	0	0	260	0	0	-	-	-	42	60	26
5333	1 2 3	-	-	-	200	40	300	140	0	290	200	0	140	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	52	36		
5352	2 3 1	-	-	-	200	56	350	-	-	-	200	0	120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	46	35		
5447	3 2 1	-	-	-	180	24	195	-	-	-	175	0	88	-	-	-	-	-	-	-	-	0	70	0	-	-	-	-	-	-	-	-	20	40	51	58	27
5553	1 2 3	-	-	-	150	62	310	-	-	-	218	0	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5701	2 1 3	-	-	-	150	62	310	-	-	-	218	0	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	53	30
5858	1 3 2	-	-	-	170	33	240	-	-	-	112	0	120	-	-	-	-	-	-	-	-	0	53	420	-	-	-	-	-	-	-	-	-	37	66	28	
5950	1 3 2	380	53	360	380	53	360	180	0	200	380	0	120	161	<10	313	161	<10	98	0	41	743	0	41	0	200	0	0	250	0	0	0	23	0	76	60	20
6175	1 2 3	-	-	-	-	-	-	-	-	-	266	44	412	266	0	179	0	40	0	0	0	200	0	0	252	0	0	0	24	21	60	62	40				
6182	3 2 1	-	-	-	-	-	-	-	-	-	150	0	166	276	44	488	276	0	155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	82	24	
6233	2 3 1	150	30	350	150	30	350	-	-	-	170	42	310	170	0	170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	53	66	30		
6253	3 1 2	-	-	-	-	-	-	-	-	-	170	42	310	170	0	170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	57	25	
6448	3 2 1	-	-	-	1000	100	400	-	-	-	130	<1	120	275	40	285	275	<1	194	-	-	-	-	-	-	270	-	-	310	-	-	-	-	-	65	57	25
6456	3 1 2	-	-	-	330	39	350	-	-	-	192	<1	112	461	58	365	435	<1	162	<1	45	773	-	-	255	<1	<1	273	<1	<1	<1	<1	17	27	53	46	26
6563	2 1 3	480	28	280	192	28	280	192	<1	112	461	58	365	435	<1	162	<1	45	773	-	-	255	<1	<1	273	<1	<1	<1	<1	17	27	53	46	26			
6686	1 2 3	-	-	-	-	-	-	-	-	-	190	29	240	190	<1	140	<1	30	470	-	-	-	-	-	-	-	-	-	-	-	-	-	34	68	31		
7248	2 3 1	460	61	270	335	61	270	160	0	330	335	0	90	288	28	457	288	0	201	0	15	790	-	-	240	0	0	220	0	0	0	9	19	71	66	27	
7442	1 3 2	505	52	400	224	52	400	-	-	-	224	0	155	200	48	452	200	0	169	0	34</																

Annex 1. Results of the participating laboratories

N = number of reported results

Min = lowest reported result

Med = median value

s_{PT} = standard deviation

F+ = false pos

<= low outlier

Lower = lowest accepted value

CV = coefficient of variation

$u_{rel, m_{PT}} = \text{relative standard uncertainty of } m_{PT}$

n = results without annotation

Max = highest reported result

m_{PT} = assigned value

$U_{\text{PT}} = \text{measurement uncertainty}$

F- = false pos

> = high outlier

Inner = highest accepted value

$\text{CV} = \text{coefficient of variation}$

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False positive or false negative

Outside the acceptance lim

Results "larger than" are no

The parameter is not evaluated

The result not evaluated

$H_{\text{opt}} \geq 0.3$ s_{opt} and/or $\geq 20\%$ outliers

The result is excluded prior determinants

The result is excluded prior determin-

Annex 1. Results of the participating laboratories

Slow-growing microorganisms (cfu/ml)			Lab no.
A	B	C	
54	64	580	1131
57	60	380	1237
71	70	610	1545
-	-	-	1594
56	60	120	1611
78	57	591	1753
65	67	659	1868
-	-	-	1970
3	1	440	2001
-	-	-	2317
-	-	-	2637
53	52	341	2745
-	-	-	3055
49	70	570	3076
-	-	-	3145
62	65	430	3162
-	-	-	3587
49	63	610	3730
65	62	485	3883
61	78	631	4015
-	-	-	4288
65	58	490	4339
67	63	570	4343
-	-	-	4356
370	120	146	4418
57	54	300	4723
-	-	-	4889
-	-	-	5201
48	62	27	5220
-	-	-	5333
59	64	141	5352
80	90	570	5447
-	-	-	5553
-	-	-	5701
-	-	-	5858
90	47	1040	5950
-	-	-	6175
61	64	583	6182
-	-	-	6233
-	-	-	6253
-	-	-	6448
-	-	-	6456
53	48	560	6563
-	-	-	6686
86	77	302	7248
77	58	750	7442
-	-	-	7688
-	-	-	7728
79	72	300	7876
-	-	-	7930
102	78	32	7946
-	-	-	7962
-	-	-	8068
62	63	525	8260
68	65	520	8329
-	-	-	8380
-	-	-	8435
69	64	670	8569

Annex 1. Results of the participating laboratories

Slow-growing microorganisms (cfu/ml)			Lab no.
A	B	C	
-	-	-	8626
62	73	84	8628
-	-	-	8663
-	-	-	8742
54	63	401	8751
54	64	243	8766
65	55	430	8829
43	56	500	8862
45	43	522	8898
73	86	550	8955
48	54	573	9436
-	-	-	9524
60	69	610	9736
53	65	715	9899
42	43	475	9903
41	41	41	N
41	41	39	n
-	-	21.526	m_{PT}
-	-	4.436	s_{PT}
-	-	0.866	u_{PT}
-	-	21	CV (%)
-	-	3.2	$u_{rel,mPT}$ (%)
-	-	0	F+
-	-	0	F-
-	-	2	<
-	-	0	>
3	1	27	Min
370	120	1 040	Max
61	63	522	Med
-	-	463	m_{PT}
3	1	67	Lower
370	120	1 214	Upper

Annex 2. Z-scores of all participants

Annex 2. Z-scores of all participants

Lab no.	Coliform bacteria (MF)			E. coli (MF)			Coliform bacteria (MPN)			E. coli (MPN)			Presumptive C. perfringens			Clostridium perfringens			Moulds			Yeasts			Actinomycetes			Culturable microorganisms 22 °C, 3 days (cfu/ml)			Slow-growing microorganisms (cfu/ml)			Lab no.	
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
8626	0.297	-0.137	-0.686	-0.528	0	3.417																										8626			
8628	1.395	0.035	0.452	-1.321	0	0.037																										8628			
8663	2.779	-1.070	-0.137	3.854	0	-0.255	0.533	1.622	1.336	0.535	0	0.765	0	-0.574	0.127	0	-0.786	0	0.817	0	0	0.734	0	0							8663				
8742	-0.640	-0.970	0.452	-0.446	0	-0.559																										8742			
8751							-0.019	-0.414	0.879	-0.017	0	-0.473																			8751				
8766	0.593	0.285	-0.447	1.105	0	0.455	0.719	-0.880	0.615	0.721	0	-0.199	0	0.967	0.669				-0.214	0	0	0.583	0	0	0	0.369	-0.453	-1.100	0.567	0.283	8766				
8829	-0.372	0.202	-0.526	-0.107	0	0.455														-0.051	0	0	-0.124	0	0	0	0.369	1.878	-0.440	0.047	-0.875	8829			
8862	-0.027	0.119	0.941	0.325	0	0.455	0.416	1.050	0.516	0.418	0	0.031	0	0.379	0.669	0	0.110	0	-0.0277	0	-0.265	-0.342	1.024	0	0	2.192	0	0	0	0.369	1.385	-1.454	-1.191	-1.234	8862
8898	2.285	1.843	0.381	3.233	0	0.589	-0.119	0.880	0.920	-0.116	0	-0.277	0	-0.265	-0.342				0.817	0	0	-1.813	0	0	0	-1.632	-0.020	-0.440	0.274	0.890	8898				
8955	1.737	-0.872	1.667	1.187	0	2.275	1.214	0.707	0.934	1.216	0	1.458	0	-0.900	0.400	0	-1.093	0	-0.553	0	0	-1.109	0	0	0	-0.650	-0.342	-0.519	-1.102	-0.196	8955				
9436	-0.295	-0.137	0.250	-0.011	0	0.207	-1.282	0.166	-0.993	-1.279	0	0.441	0	-0.116	0.669				1.165	0	0	0.920	0	0	0	0.908	0.385	0.384	0.920	-0.034	9436				
9524							-0.867	0	1.105	0.152	-1.042	-0.558	0.154	0	-1.255	0	1.030	-1.931				-0.709	0	0	0.564	0	0	0	0.908	0.948	-0.519	0.709	2.012	9524	
9736							1.014	0.072	-0.127	1.016	0	0.182	0	0.712	-3.326				1.165	0	0	0.920	0	0	0	0.908	0.385	0.384	0.920	-0.034	9736				
9899	-0.387	0.839	-0.291	-0.127	0	0.589	-0.044	0.707	0.200	-0.042	0	0.330	0	0.310	1.002				0.505	0	0	1.229	0	0	0	0.462	-1.047	-1.364	-1.102	2.790	9899				
9903	-0.805	0.202	-0.291	-0.304	0	1.351																									0.060	9903			

- $|z| \geq 3,0$ ("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: <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