

Proficiency testing Drinking water Microbiology

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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)
LTLNB	Lactose tryptone lauryl sulphate broth (SS 028167)
m-FC	m-FC Agar (SS 028167)
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

Yeasts

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 square root transformed 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 78 participants; 39 in Sweden, 37 in European countries, and two outside of Europe. Of the 73 participants that reported results, 30 (41 %) provided at least one result that received an annotation.

Individual results are listed in Annex 1. 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>Klebsiella pneumoniae</i> <i>Kluyveromyces marxianus</i> <i>Phialophora malorum</i> <i>Macroccoccus caseolyticus</i>			<i>Enterobacter aerogenes</i> <i>Clostridium perfringens</i> <i>Pichia kudriavzevii</i> <i>Cladosporium cladosporioides</i> <i>Aeromonas caviae</i>			<i>Escherichia coli</i> <i>Citrobacter freundii</i> <i>Clostridium bifermentans</i> <i>Sphingomonas species</i> <i>Streptomyces species</i> <i>Staphylococcus saprophyticus</i>					
Analysis	Target organism	N	F	X	Target organism	N	F	X	Target organism	N	F	X
Coliform bacteria (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	51	0	1	<i>E. aerogenes</i> (<i>A. caviae</i>)	50	3	1	<i>E. coli</i> <i>C. freundii</i>	52	1	4
Susp. thermotol. coliform bact. (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	19	0	0	(<i>E. aerogenes</i>)	20	0	0	<i>E. coli</i>	20	0	0
<i>E. coli</i> (MF)	<i>E. coli</i>	50	1	0	-	51	1	0	<i>E. coli</i>	51	0	5
Coliform bacteria (MPN)	<i>E. coli</i> <i>K. pneumoniae</i>	44	0	1	<i>E. aerogenes</i>	43	0	1	<i>E. coli</i> <i>C. freundii</i>	45	1	1
<i>E. coli</i> (MPN)	<i>E. coli</i>	44	1	1	-	45	0	0	<i>E. coli</i>	45	1	0
Presumptive <i>C. perfringens</i>	-	41	5	0	<i>C. perfringens</i>	41	1	1	<i>C. bifermentans</i>	41	0	0
<i>C. perfringens</i>	-	26	4	0	<i>C. perfringens</i>	27	2	2	(<i>C. bifermentans</i>)	27	5	0
Moulds	<i>P. malorum</i>	34	1	1	<i>C. cladosporioides</i>	35	0	2	-	35	1	0
Yeasts	<i>K. marxianus</i>	34	0	2	<i>P. kudriavzevii</i>	35	0	2	-	35	1	0
Actinomycetes	-	27	0	0	-	27	0	0	<i>Streptomyces sp.</i>	27	0	0
Culturable microorganisms 22 °C, 3 days	<i>M. caseolyticus</i>	68	1	3	<i>A. caviae</i> <i>E. aerogenes</i>	69	0	5	<i>S. saprophyticus</i>	69	1	2
Slow-growing bacteria	<i>M. caseolyticus</i>	40	0	2	<i>A. caviae</i> <i>E. aerogenes</i>	41	0	3	<i>Sphingomonas</i> <i>S. saprophyticus</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 strains of *E. coli* and *K. pneumoniae* were target organisms. Both strains form typical colonies with a metallic sheen on m-Endo Agar LES (LES). On Chromocult Coliform Agar (CCA), *E. coli* and *K. pneumoniae* form blue and pink colonies, respectively. The strains possess the enzyme β -galactosidase and are detected as coliform bacteria with Colilert/Colilert-18.

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

In total, 51 participants reported results for membrane filtration (MF) methods. One low outlier was reported.

For most probable number (MPN) methods, 44 participants reported results. One low outlier was reported.

Sample B

The strain of *E. aerogenes* 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 MF methods. Three false-negative results and one high outlier were reported.

The participants who reported false-negative results all reported suspected coliform bacteria, which they assessed as non-coliform bacteria after confirmation.

The high outlier may be due to the inclusion of the false-positive *A. caviae* as the concentration is reasonable.

For MPN methods, 43 participants reported results. One low outlier was reported.

Sample C

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

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

In total, 52 participants reported results for MF methods. One false-negative result as well as one high and three low outliers were reported.

For MPN methods, 45 participants reported results. One false-negative result and one low outlier were reported.

General remarks

For MF methods, most participants (55 %) followed (EN) ISO 9308-1:2014 using the enzyme-based chromogenic medium CCA. CCA is suitable for waters with low bacterial background flora due to the low selectivity of the medium. On CCA, β -D-galactosidase positive (pink to red) colonies are counted as presumptive coliform bacteria. β -D-galactosidase and β -D-glucuronidase positive (dark blue to violet) colonies are counted as *E. coli*. Total coliform bacteria are the sum of oxidase-negative presumptive coliform bacteria and *E. coli*. ISO 9308-1:2014 was last reviewed by ISO in 2019 and remains current. An amendment of the incubation time and performance testing of CCA is available (ISO 9308-1:2014/Amd 1:2016).

SS 028167 and SFS 3016 are Nordic national standards using LES. On LES, coliform bacteria form red colonies with a metallic sheen due to the production of aldehyde from the fermentation of lactose. The presumptive coliform bacteria are confirmed by a negative oxidase test.

MPN methods are based on the growth of target organisms in a liquid medium and calculation of the MPN of organisms by reference to MPN tables. For MPN methods, most participants (86%) followed (EN) ISO 9308-2:2012 using Colilert-18. ISO 9308-2:2012 was last reviewed by ISO in 2023 and remains current. As with CCA, Colilert is based on the activity of β -D-galactosidase. β -D-galactosidase cleave ortho-nitrophenol galactoside (ONPG) and change the coloration of the wells to yellow. Some participants reported the use of Colilert-18 according to the manufacturers' instructions and are referred as "Colilert-18" in Table 3 and Figure 1.

For all results, the m_{PT} for MPN methods was higher compared to the MF-methods, particularly for sample B.

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	51	50	57	9	0	1	0	50	46	1174	15	3	0	1	52	47	39	17	1	3	1
ISO 9308-1:2014	28	27	57	9	0	1	0	26	24	1151	14	1	0	1	28	26	39	20	1	0	1
SFS 3016	11	11	56	8	0	0	0	12	12	1100	19	0	0	0	12	10	36	11	0	2	0
SS 028167	10	10	58	9	0	0	0	10	8	1090	22	2	0	0	10	10	47	10	0	0	0
ISO 9308-1:1990	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	1	-	-	0	1	0
Medium																					
All results	51	50	57	9	0	1	0	50	46	1174	15	3	0	1	52	47	39	17	1	3	1
Chromocult Coliform Agar	27	26	57	9	0	1	0	25	23	1152	12	1	0	1	27	25	39	20	1	0	1
m-Endo Agar LES	24	24	56	9	0	0	0	25	23	1100	21	2	0	0	25	22	40	12	0	3	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

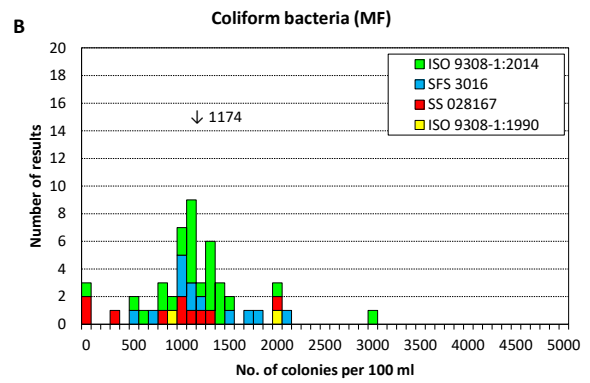
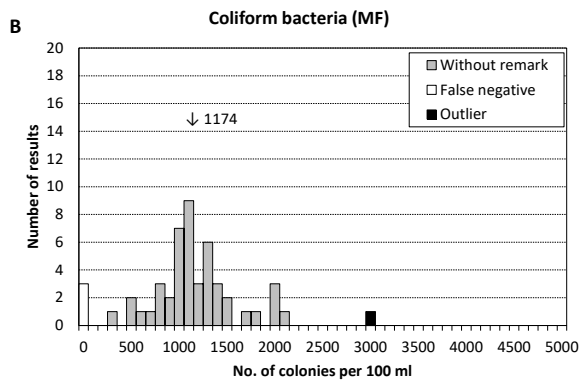
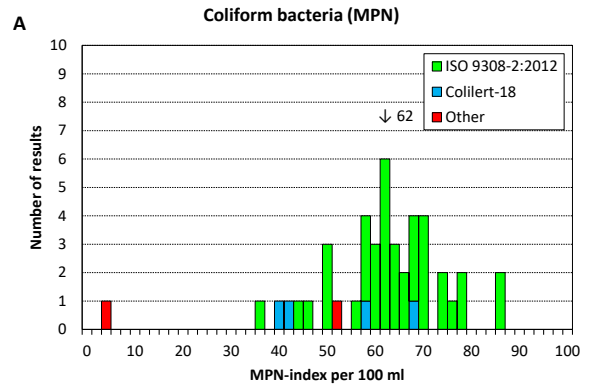
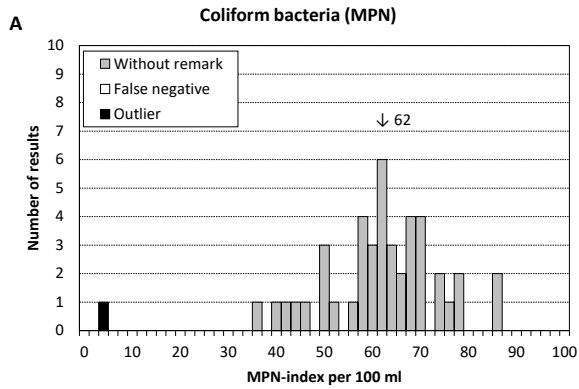
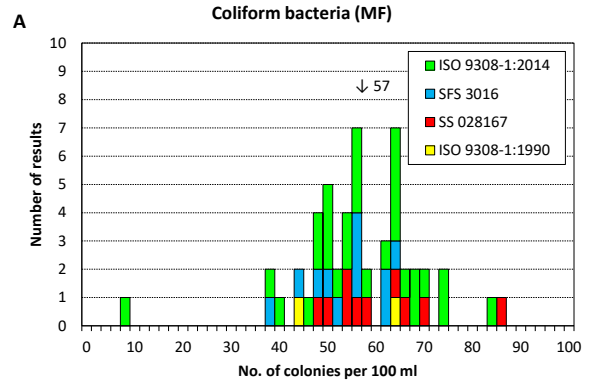
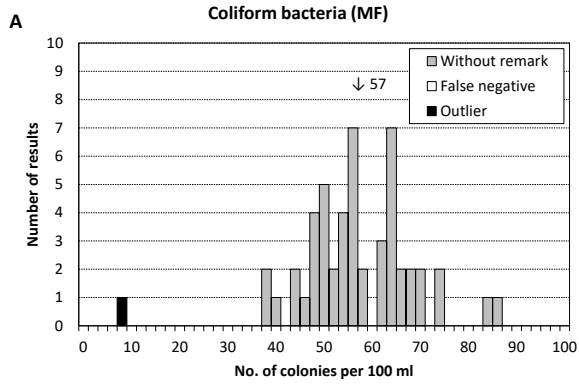
Table 3. Results from analysis of coliform bacteria with MPN methods.

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	44	43	62	9	0	1	0	43	42	1330	11	0	1	0	45	43	45	16	1	1	0
ISO 9308-2:2012	38	38	63	9	0	0	0	39	39	1301	11	0	0	0	39	39	45	14	0	0	0
Colilert-18	4	4	-	-	0	0	0	2	2	-	-	0	0	0	4	3	-	-	0	1	0
Other*	2	1	-	-	0	1	0	2	1	-	-	0	1	0	2	1	-	-	1	0	0

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

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

* The group Other include reporting of a multiple tube method based on lactose fermentation and the use of Colilert.



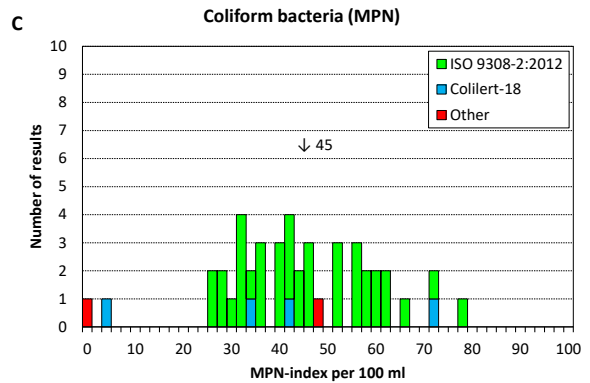
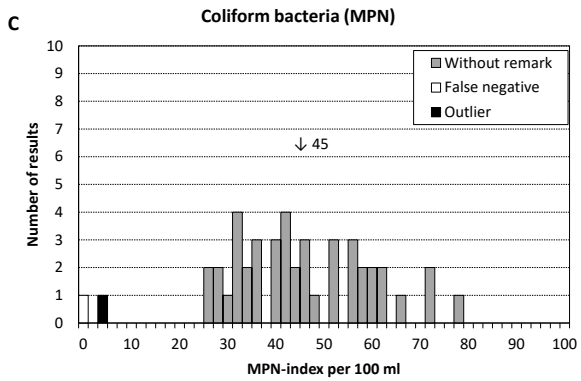
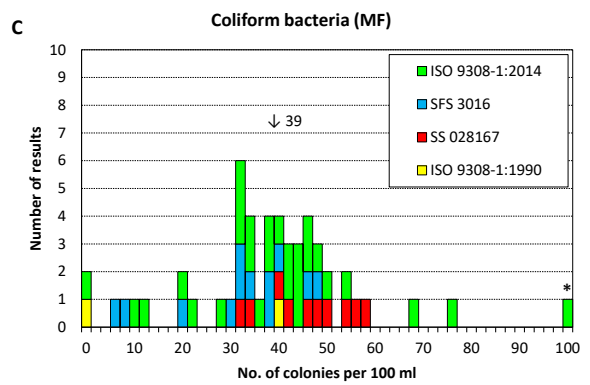
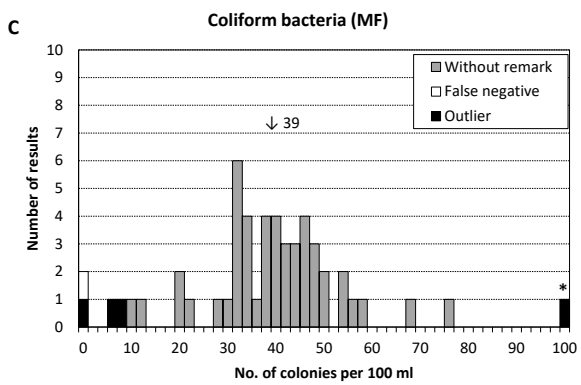
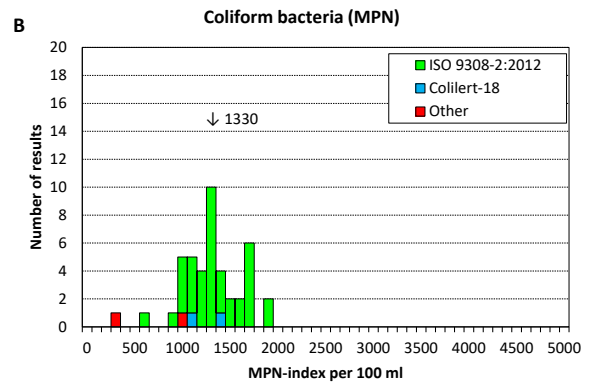
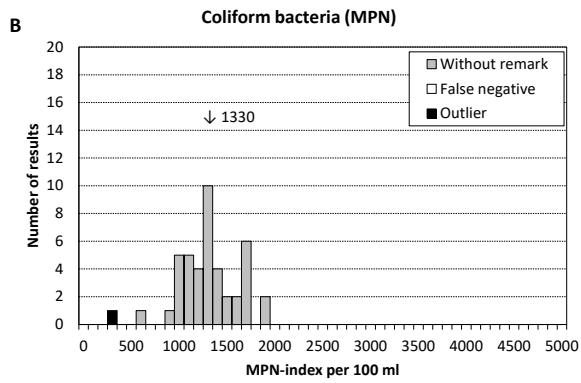


Figure 1. Results from analysis of coliform bacteria

Suspected thermotolerant coliform bacteria

Sample A

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, 19 participants reported results.

Sample B

No thermotolerant coliform bacterium was present in the sample. However, on m-FC Agar, the strain of *E. cloacae* may grow as suspected thermotolerant coliform bacteria at 44/44.5 °C with small colonies.

In total, 20 participants reported results.

Sample C

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

In total, 20 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, 20 participants reported results. Of these, 17 participants (85 %) 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 and ISO 9308-1 (old edition) 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	19	19	52	-	-	-	-	20	20	0	-	-	-	-	20	20	14	-	-	-	-
SS 028167	7	7	52	-	-	-	-	7	7	0	-	-	-	-	7	7	21	-	-	-	-
SFS 4088	5	5	56	-	-	-	-	6	6	0	-	-	-	-	6	6	17	-	-	-	-
Other	3	3	-	-	-	-	-	3	3	-	-	-	-	-	3	3	-	-	-	-	-
ISO 9308-1 (old edition)	2	2	-	-	-	-	-	2	2	-	-	-	-	-	2	2	-	-	-	-	-
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.

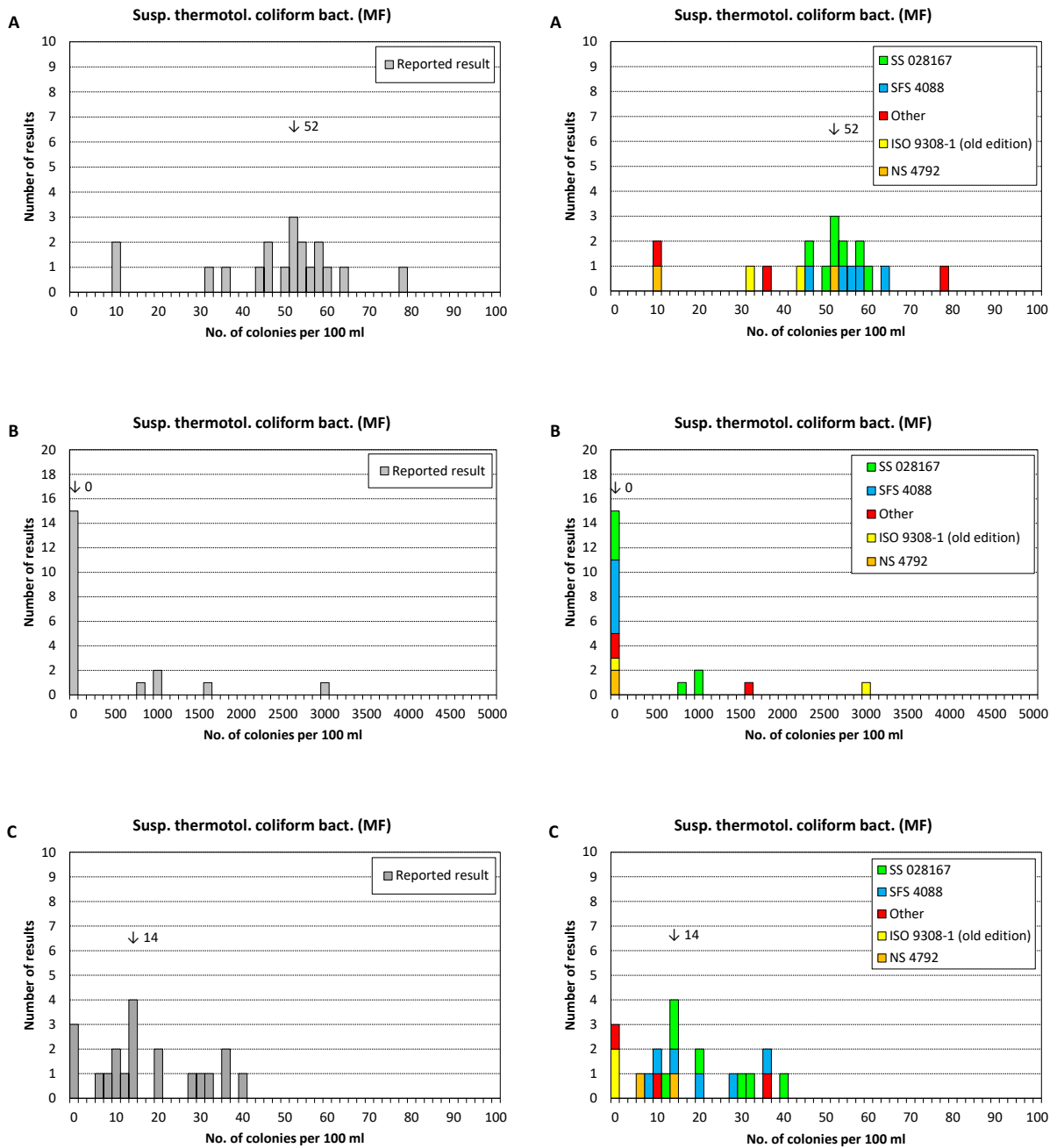


Figure 3. Results from analysis of *suspected thermotolerant coliform bacteria*

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 Colilert/Colilert-18. The strain is positive for indole production and it produces gas in Lactose-Tryptone-Lauryl Sulphate Broth (LTLSB).

In total, 50 participants reported results for MF methods. One false negative result was reported.

For MPN methods, 44 participants reported results. One false negative result and one low outlier were reported.

Sample B

No target organism was present in the sample.

In total, 51 participants reported results for MF methods. One false-positive result was reported.

For MPN methods, 45 participants reported results. All reported results were correct negative.

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 but produces no gas in LTLSB.

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

For MPN methods, 45 participants reported results. One false-negative result was reported.

General remarks

Most participants followed (EN) ISO 9308-1:2014, Nordic national standards (see table 4) and/or (EN) ISO 9308-2:2012. 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. Since ISO 9308-1:2014 only requires expression of β -D-glucuronidase, some participants have modified their standard accordingly. Depending on the method, tests for gas production, 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	50	49	13	19	1	0	0	51	50	-	-	1	-	-	51	46	26	16	0	4	1
ISO 9308-1:2014	30	29	12	19	1	0	0	30	29	-	-	1	-	-	30	27	29	11	0	2	1
SFS 3016, modified	8	8	13	23	0	0	0	9	9	-	-	0	-	-	9	8	23	14	0	1	0
SS 028167, modified	6	6	13	14	0	0	0	6	6	-	-	0	-	-	6	6	29	18	0	0	0
SS 028167	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
ISO 9308-1 (old edition)	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	1	-	-	0	1	0
SFS 4088	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

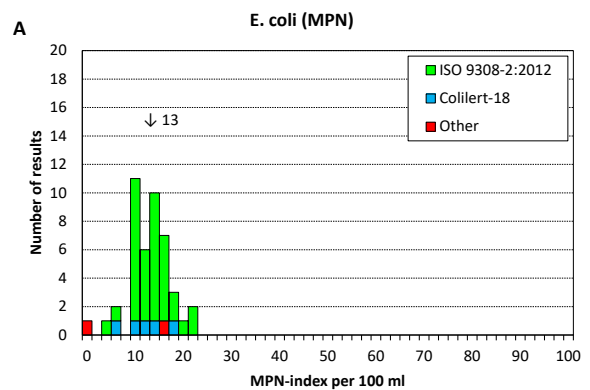
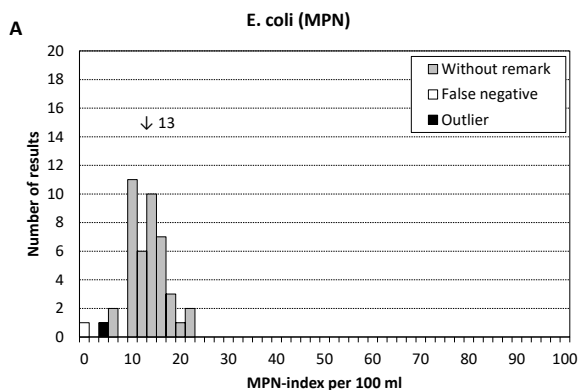
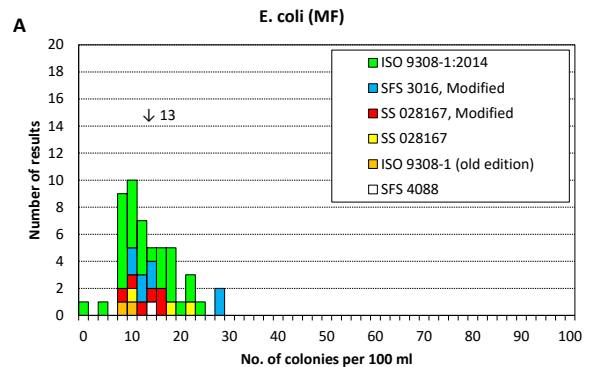
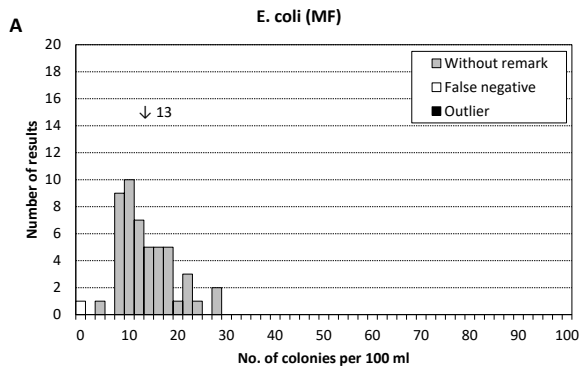
Table 5. Results from analysis of *Escherichia coli* with MPN methods.

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	44	42	13	13	1	1	0	45	45	-	-	0	-	-	45	44	32	14	1	0	0
ISO 9308-2:2012	37	36	14	13	0	1	0	38	38	-	-	0	-	-	38	38	32	14	0	0	0
Colilert-18	5	5	12	19	0	0	0	5	5	-	-	0	-	-	5	5	27	14	0	0	0
Other*	2	1	-	-	1	0	0	2	2	-	-	0	-	-	2	1	-	-	1	0	0

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

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

* The group Other includes reporting of a multiple tube method based on lactose fermentation and the use of Colilert-24.



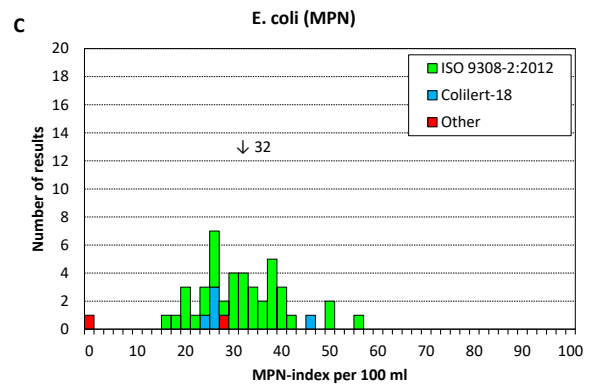
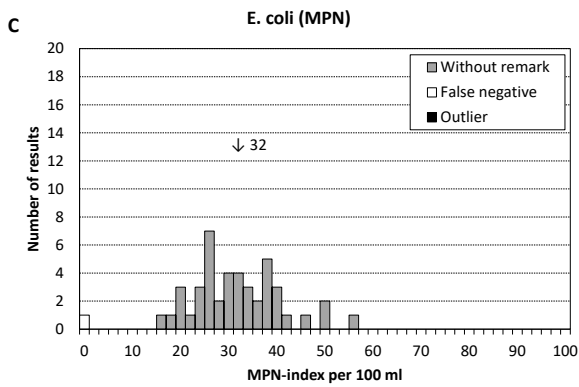
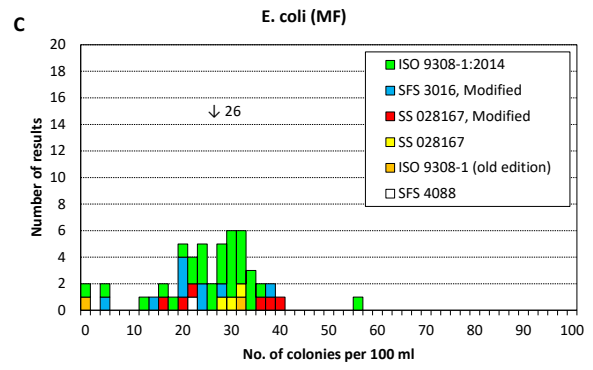
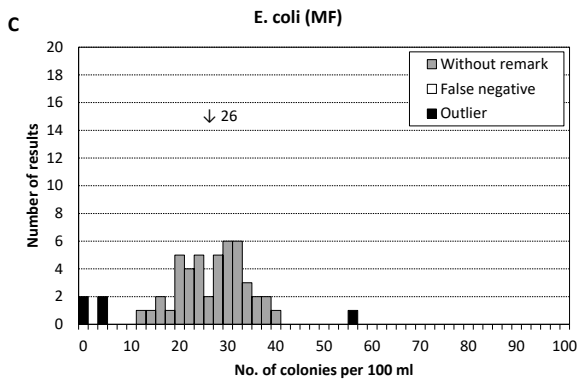


Figure 2. Results from analysis of *Escherichia coli*

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*. Five false positive-results were reported.

For *C. perfringens*, 26 participants reported results. Four 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*. One false-negative result and one low outlier were reported.

For *C. perfringens*, 27 participants reported results. Two false-negative results as well as one high and one low outlier were reported. The false-negative results may be due to participants mixing up the samples since the same participants have false-positive results as well.

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*.

In the Swedish Food Agency's quality control, the homogeneity was approved for presumptive *C. perfringens* and the concentration mean was 6607 cfu 100 ml⁻¹. In this PT-round, the results for presumptive *C. perfringens* ranged between 0–8300 cfu 100 ml⁻¹ and were almost evenly dispersed within this range. This resulted in a very high standard deviation and consequently an unrealistically wide acceptance interval and uninformative z-scores. As a consequence, the results are not evaluated.

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

For *C. perfringens*, 27 participants reported results. Five 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* and no sample pasteurisation should be done before analyses. Participants may report presumptive (non-confirmed) or confirmed *C. perfringens*.

Most participants followed (EN) ISO 14189, which was last reviewed by ISO in 2019 and remains current. ISO 14189 is a TSC-based method that enables the detection and enumeration of *C. perfringens* in different types of water. 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. As the confirmation step requires the use of a carcinogenic reagent, some laboratories do not perform the acid phosphatase test and interpret presumptive *C. perfringens* as positive results.

Three participants reported the use of TSC following the draft standard ISO/CD 6461-2:2002, with or without modifications. One participant reported following Council Directive 98/83/EC [2], which is based on m-CP agar.

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

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	Med*	CV	F	<	>
All results	41	36	-	-	5	-	-	41	39	30	14	1	1	0	41	41	3500	-	-	-	-
ISO 14189:2013	35	30	-	-	5	-	-	35	33	29	13	1	1	0	35	35	3800	-	-	-	-
ISO/CD 6461-2:2002, modified	3	3	-	-	0	-	-	3	3	-	-	0	0	0	3	3	-	-	-	-	-
Other**	2	2	-	-	0	-	-	2	2	-	-	0	0	0	2	2	-	-	-	-	-
ISO/CD 6461-2:2002	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	-	-	-

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

Med = Median value in cfu 100 ml⁻¹

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

** The group Other include reporting of the use of TSC agar (unknown method) and m-CP agar with confirmation by VITEK®.

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	22	-	-	4	-	-	27	23	33	16	2	1	1	27	22	-	-	5	-	-
ISO 14189:2013	22	18	-	-	4	-	-	22	18	29	13	2	1	1	22	19	-	-	3	-	-
ISO/CD 6461-2, modified	2	2	-	-	0	-	-	3	3	-	-	0	0	0	3	2	-	-	1	-	-
EU Directive 1998 (m-CP Agar)	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	1	-	-	0	-	-
ISO/CD 6461-2:2002	1	1	-	-	0	-	-	1	1	-	-	0	0	0	1	0	-	-	1	-	-

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

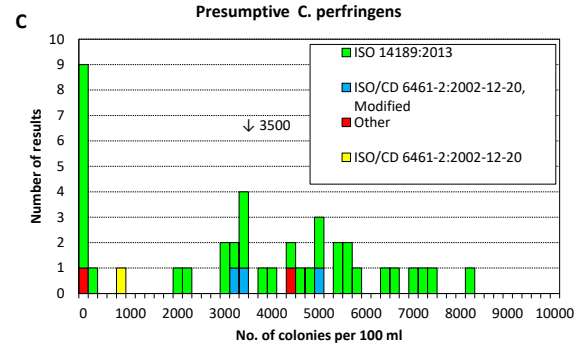
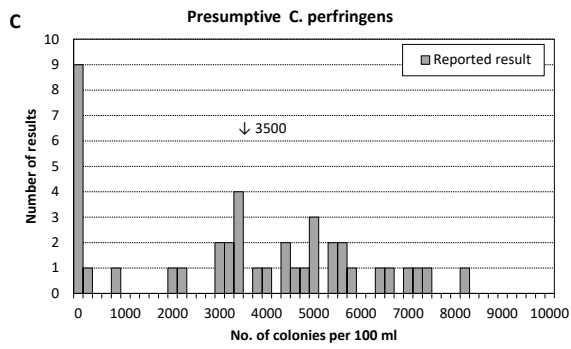
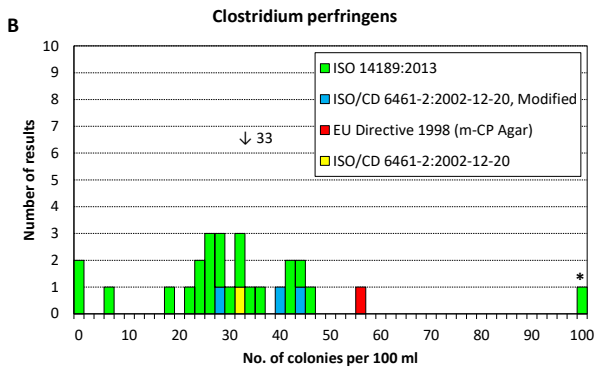
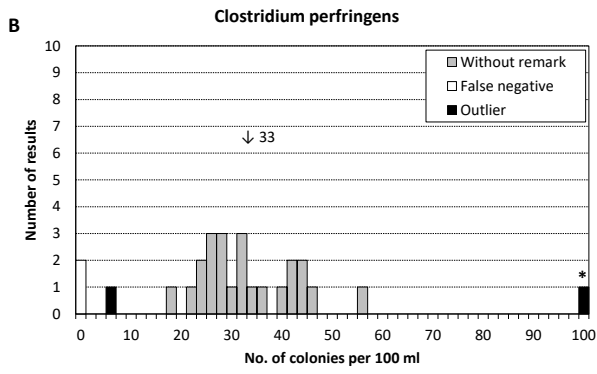
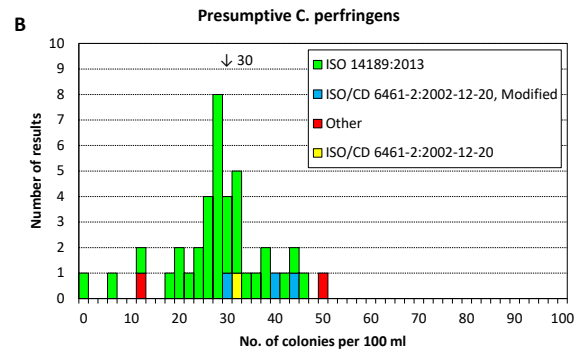
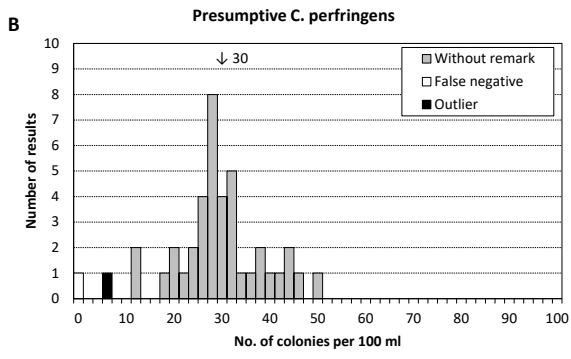


Figure 4. Results from analysis of presumptive and confirmed *Clostridium perfringens*.

Moulds and yeasts

Sample A

The strains of *P. malorum* and *K. marxianus* were target organisms for moulds and yeasts respectively.

In total, 34 participants reported results for moulds. One false-negative result and one low outlier were reported.

For yeasts, 34 participants reported results. One high and one low outlier were reported.

Sample B

The strains of *C. cladosporioides* and *P. kudriavzevii* were target organisms for moulds and yeasts respectively.

The concentrations of moulds and yeasts were low; m_{PT} was 9 cfu 100 ml⁻¹ and 3 cfu 100 ml⁻¹, respectively. A reported result of <1 cfu 100 ml⁻¹ was included in the expected results range and is considered acceptable. Z-scores lower than -3 should be treated as acceptable.

In total, 35 participants reported results for moulds. Two high outliers were reported.

For yeasts, 35 participants reported results. Two high outliers were reported.

Sample C

No target organism was present in the sample.

In total, 35 participants reported results for moulds. One false-positive result was 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.

In this PT round, results reported using DRBC are separated in tables 9–10 and figure 5. Otherwise, participants who reported SS 028192, SFS 5507 or NS (SS) 028192 have reported the use of Rose Bengal agar including Cooke Rose Bengal agar base, Rose Bengal agar base, Rose Bengal agar, Rose Bengal Chloramphenicol agar.

Table 9. Results from analysis of moulds.

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	34	32	77	17	1	1	0	35	33	9	31	0	0	2	35	34	-	-	1	-	-
SS 028192	19	19	80	12	0	0	0	19	19	7	28	0	0	0	19	19	-	-	0	-	-
Other	6	4	-	-	1	1	0	7	6	11	53	0	0	1	7	6	-	-	1	-	-
SS 028192 (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
NMKL 98:2005, modified (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
SFS 5507 (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
SFS 5507	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
NS (SS) 028192	1	1	-	-	0	0	0	1	0	-	-	0	0	1	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

*The group Other includes reporting of the use of Malt Extract (ME) agar, Rose Bengal Agar according to ISO 21527, Oxytetracyclin Glucose Yeast Extract Agar (OGYE) and Sabouraud Dextrose Agar with chloramphenicol.

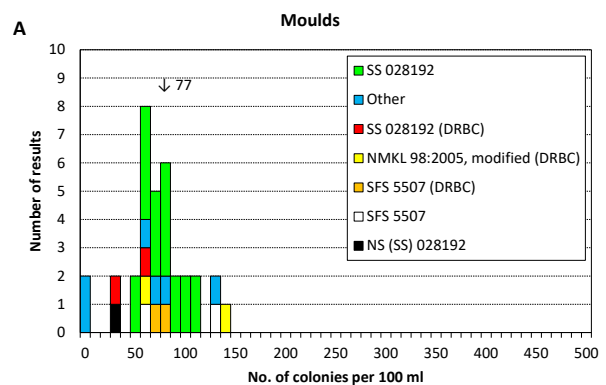
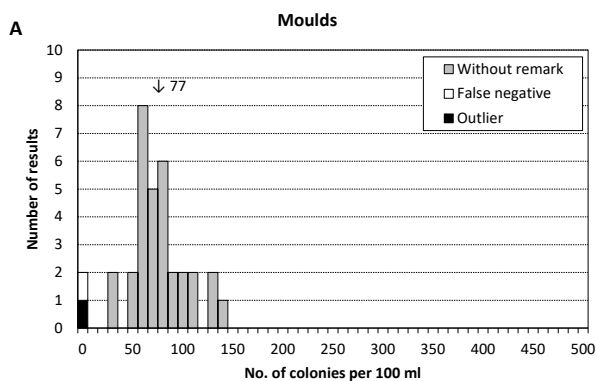
Table 10. Results from analysis of yeasts.

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	34	32	80	15	0	1	1	35	33	3	33	0	0	2	35	34	-	-	1	-	-
SS 028192	19	19	78	15	0	0	0	19	19	2	66	0	0	0	19	19	-	-	0	-	-
Other*	6	4	-	-	0	1	1	7	5	4	92	0	0	2	7	6	-	-	1	-	-
SS 028192 (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
NMKL 98:2005, modified (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
SFS 5507 (DRBC)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
SFS 5507	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
NS (SS) 028192	1	1	-	-	0	0	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

*The group Other include reporting of the use of Malt Extract (ME) agar, Rose Bengal Agar according to ISO 21527, Oxytetracyclin Glucose Yeast Extract Agar (OGYE) and Sabouraud Dextrose Agar with chloramphenicol.



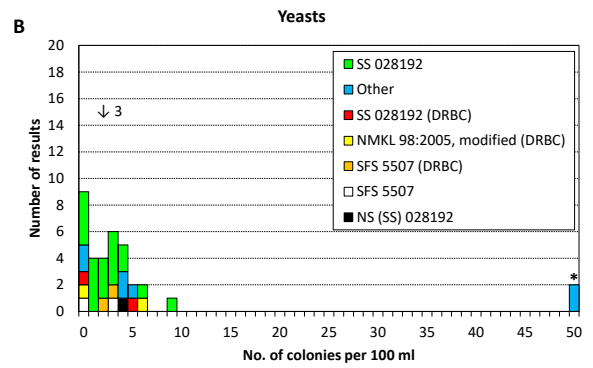
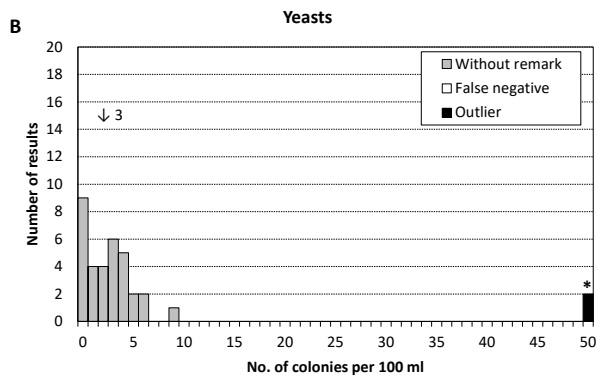
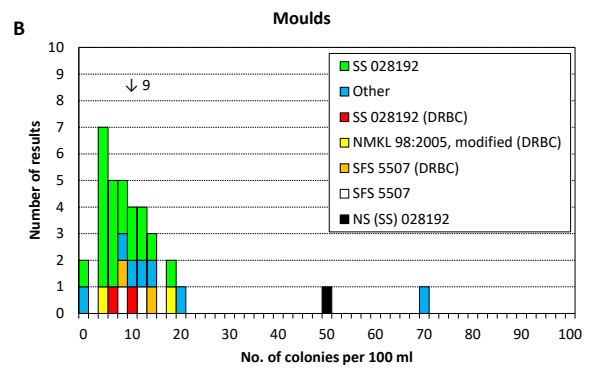
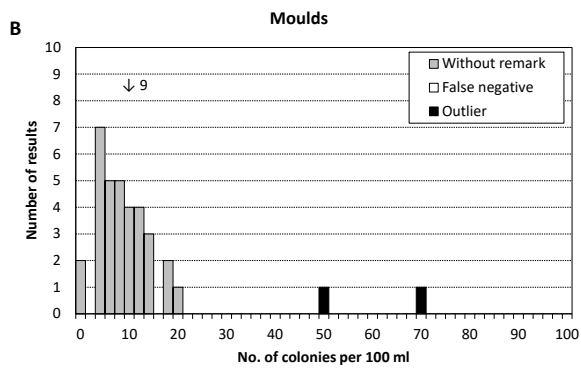
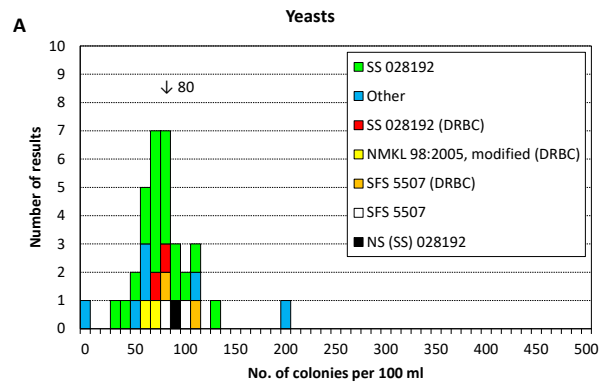
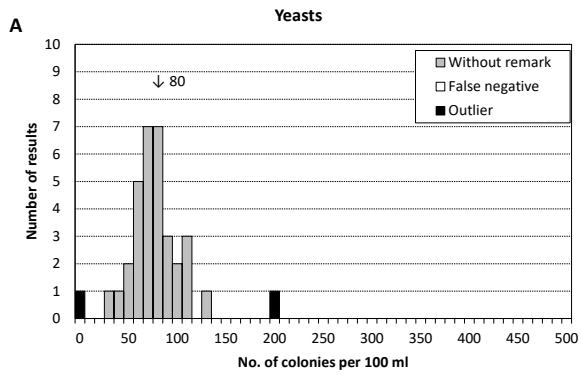


Figure 5. Results from analysis of moulds and yeasts.

Actinomycetes

Sample A

No target organism was present in the sample.

In total, 27 participants reported results. No false-positive results were reported.

Sample B

No target organism was present in the sample.

In total, 27 participants reported results. No false-positive results were reported.

Sample C

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

In total, 27 participants reported results.

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. Five participants used other methods, with natamycin as the selective substance.

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	27	27	-	-	0	-	-	27	27	-	-	0	-	-	27	27	149	11	0	0	0	0
SS 028212	22	22	-	-	0	-	-	22	22	-	-	0	-	-	22	22	149	12	0	0	0	0
Other	5	5	-	-	0	-	-	5	5	-	-	0	-	-	5	5	160	9	0	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

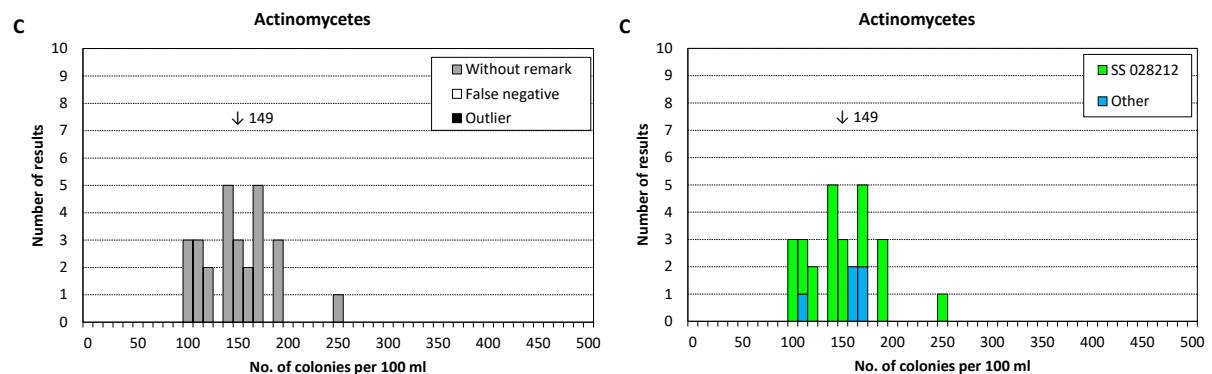


Figure 6. Results from analysis of actinomycetes.

Culturable microorganisms, 72 hours incubation at 22 °C

Sample A

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

In total, 68 participants reported results. One false-negative result as well as one high and two low outliers were reported.

Sample B

The strains of *E. aerogenes* and *A. hydrophila* were main target organisms.

In total, 69 participants reported results. Three low and two high outliers were reported.

Sample C

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

In total, 69 participants reported results. One false-negative result as well as one high and one low outlier were reported.

General remarks

Sixty-six out of 68 participants followed (EN) ISO 6222:1999, which is based on a pour-plate method with Yeast extract Agar (YEA). ISO 6222:1999 was last reviewed by ISO in 2021 and remains current.

Fifty-six participants included both moulds and yeasts in the reported results. Four participants included only yeasts and nine participants did not include either mould or yeast.

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	68	64	45	10	1	2	1	69	64	31	13	0	3	2	69	66	18	12	1	1	1
ISO 6222	66	62	45	9	1	2	1	67	62	31	11	0	3	2	67	64	18	12	1	1	1
Other*	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Magnification																					
All results	68	64	45	10	1	2	1	69	64	31	13	0	3	2	69	66	18	12	1	1	1
8-11.9	25	25	43	9	0	0	0	25	24	35	9	0	0	1	25	25	18	11	0	0	0
5-7.9	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
2-4.9	9	7	52	7	0	1	1	9	8	26	12	0	1	0	9	9	18	11	0	0	0
1.1-1.9	16	16	45	11	0	0	0	16	14	30	10	0	1	1	16	15	16	14	0	1	0
None	14	12	46	7	1	1	0	15	14	27	13	0	1	0	15	13	17	12	1	0	1

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 group Other includes reporting of Standard Methods, 9215 B [3] and 3M™ Petrifilm™ Aerobic Count Plate.

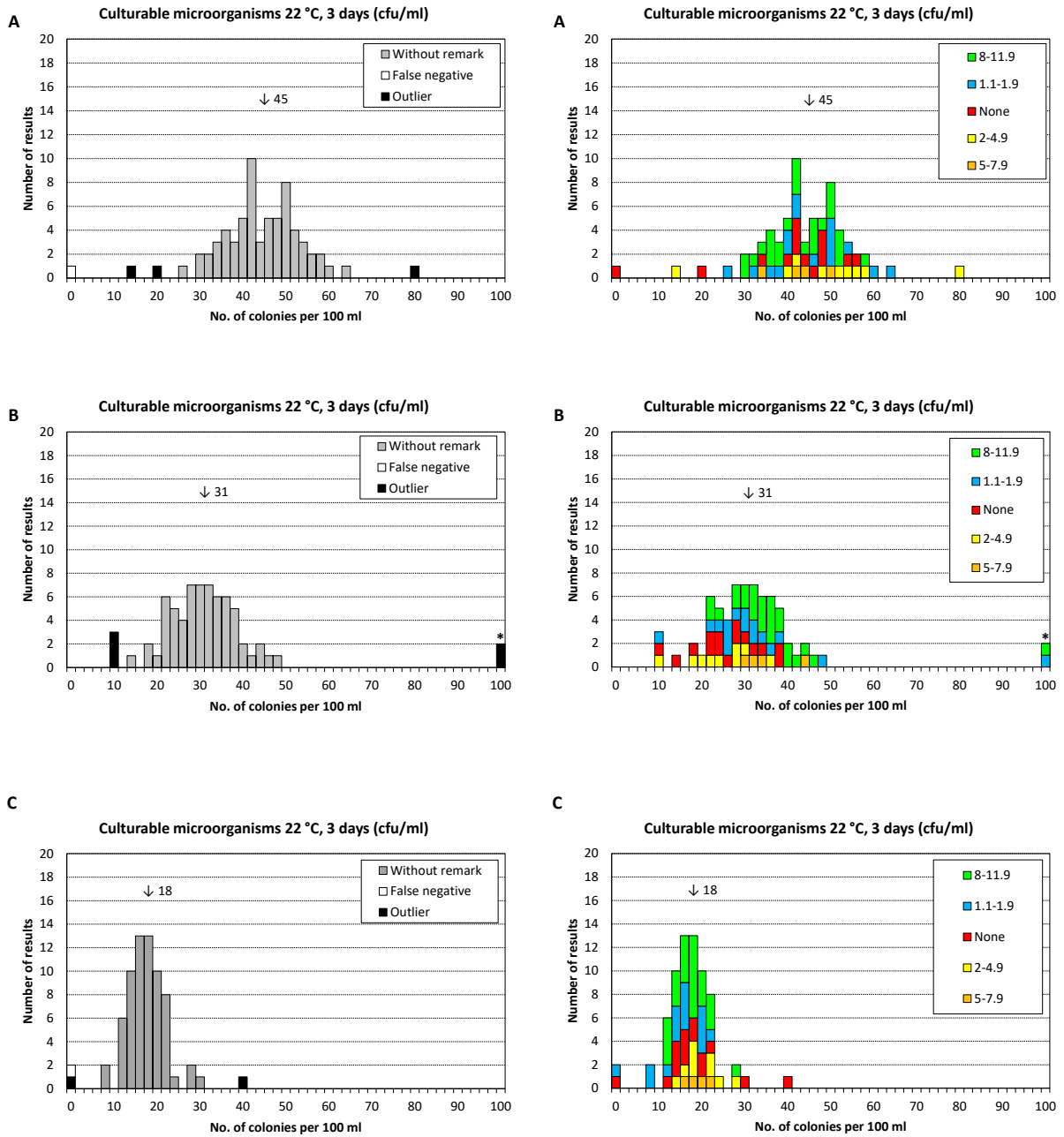


Figure 8. Results from analysis of culturable microorganisms, 72 hours incubation at 22 °C

Slow-growing bacteria 22 °C, 7 days

Sample A

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

In total, 40 participants reported results. One high and one low outlier were reported.

Sample B

No specific slow-growing bacterium was included in the sample but the strains of *E. aerogenes* and *A. hydrophila* form colonies on YEA and R2A at 22 °C after 7 days.

In total, 41 participants reported results. One high and two low outliers were reported.

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 a 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.

Sample C included a slow-growing *Sphingomonas* sp. bacterium. It often forms small colonies, and magnification is usually needed.

Most participants used YEA and five 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							
	<i>N</i>	<i>n</i>	<i>m_{PT}</i>	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m_{PT}</i>	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m_{PT}</i>	<i>CV</i>	<i>F</i>	<	>	
All results	40	38	46	12	0	1	1	41	38	34	12	0	2	1	41	39	70	10	0	2	0	
ISO 6222:1999	36	34	47	10	0	1	1	36	33	36	10	0	2	1	36	34	70	10	0	2	0	
Other	4	4	-	-	0	0	0	5	5	30	16	0	0	0	5	5	74	8	0	0	0	
Medium																						
All results	40	38	46	12	0	1	1	41	38	34	12	0	2	1	41	39	70	10	0	2	0	
YEA	31	29	47	9	0	1	1	31	29	35	8	0	1	1	31	31	74	10	0	0	0	
Other	5	5	50	6	0	0	0	5	4	-	-	0	1	0	5	3	-	-	0	2	0	
R2A	4	4	-	-	0	0	0	5	5	36	19	0	0	0	5	5	73	12	0	0	0	
Magnification																						
All results	40	38	46	12	0	1	1	41	38	34	12	0	2	1	41	39	70	10	0	2	0	
5-11.9	25	24	47	9	0	0	1	25	24	36	7	0	0	1	25	24	75	6	0	1	0	
1.1-4.9	8	7	50	16	0	1	0	9	8	30	16	0	1	0	9	9	62	17	0	0	0	
None	4	4	-	-	0	0	0	4	3	-	-	0	1	0	4	3	-	-	0	1	0	
Other	3	3	-	-	0	0	0	3	3	-	-	0	0	0	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

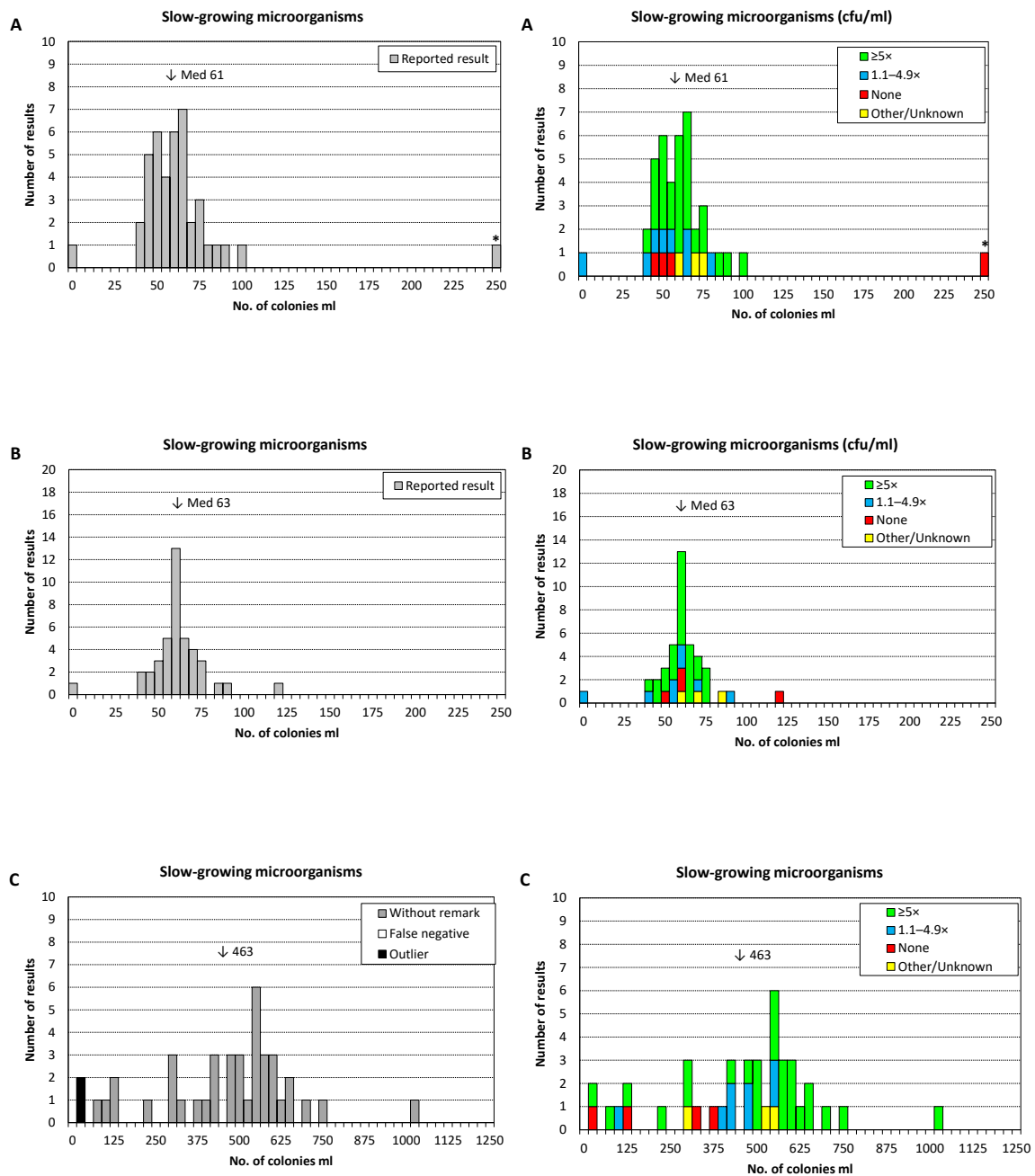


Figure 9. Results from analysis of slow-growing bacteria

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 [4].

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 properties of a box plot are shown in figure 10.

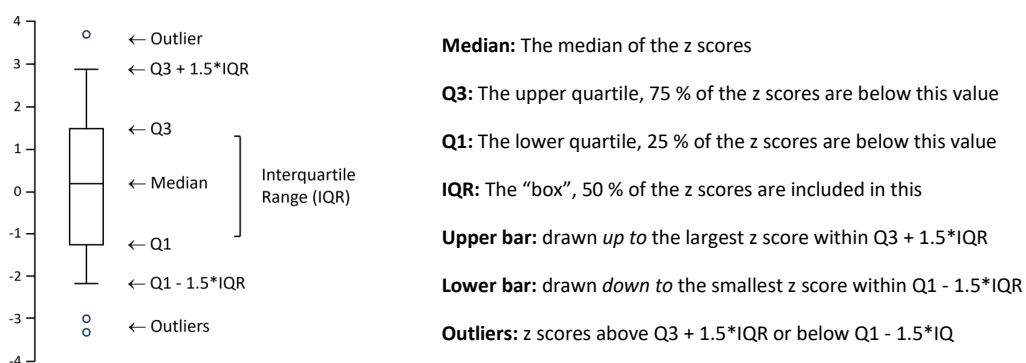
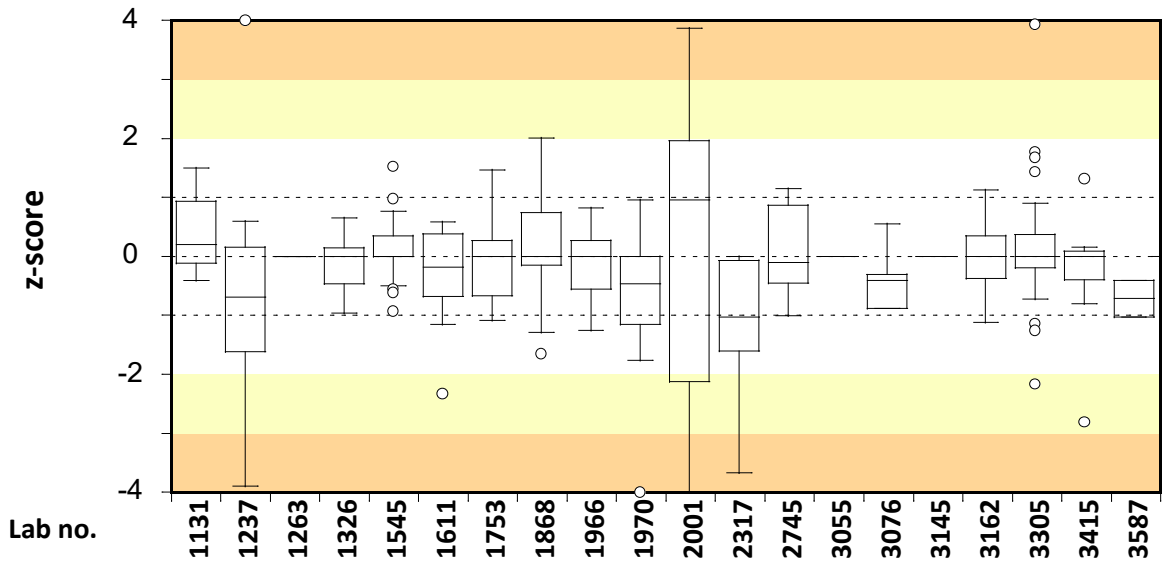
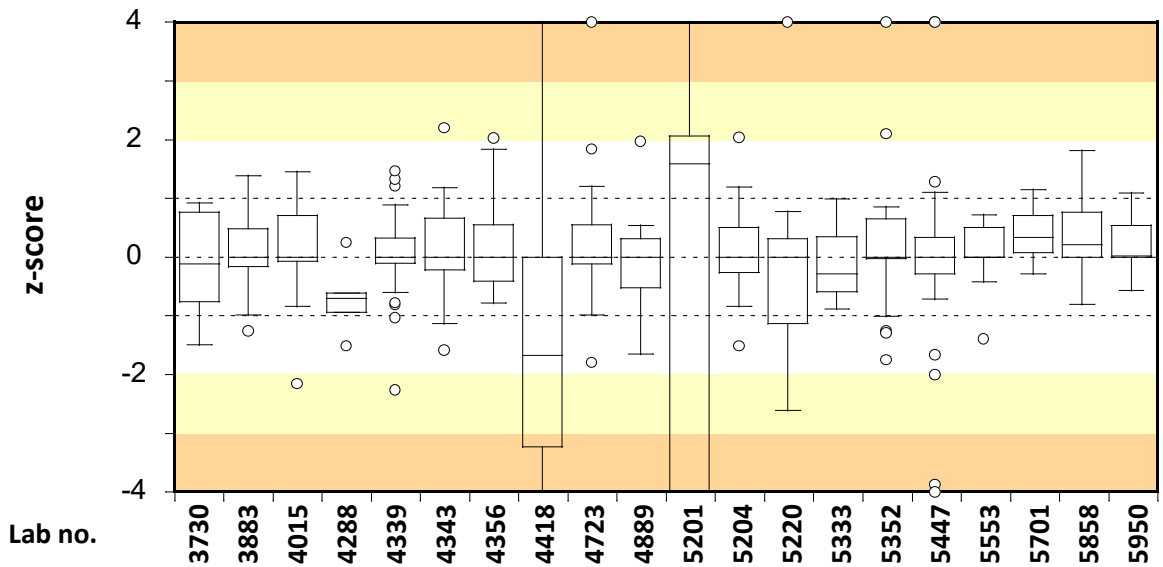


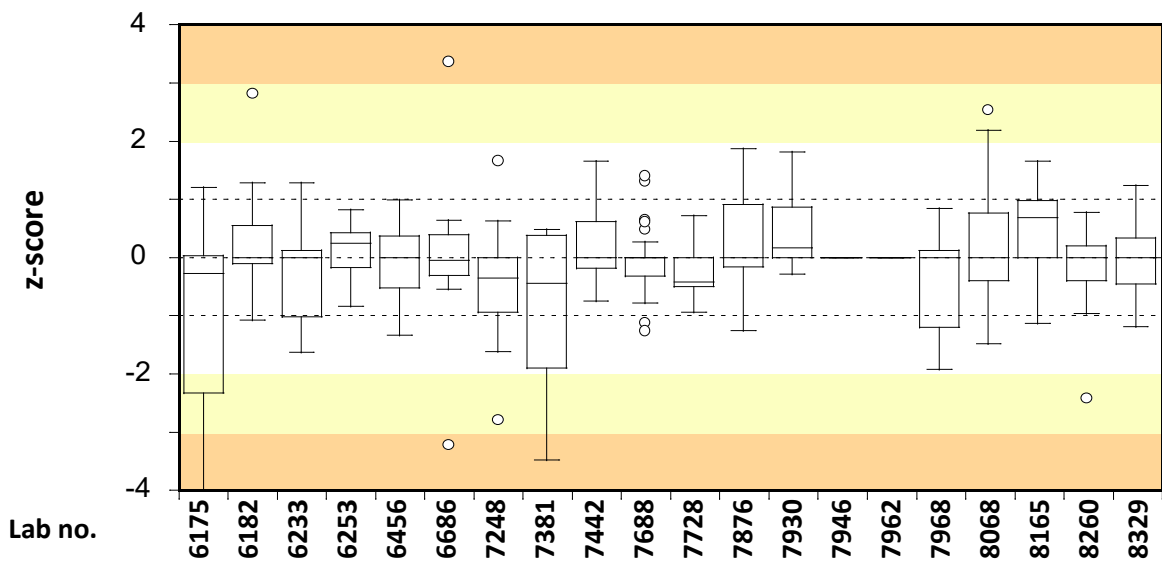
Figure 10. Schematic explanation of a box plot.



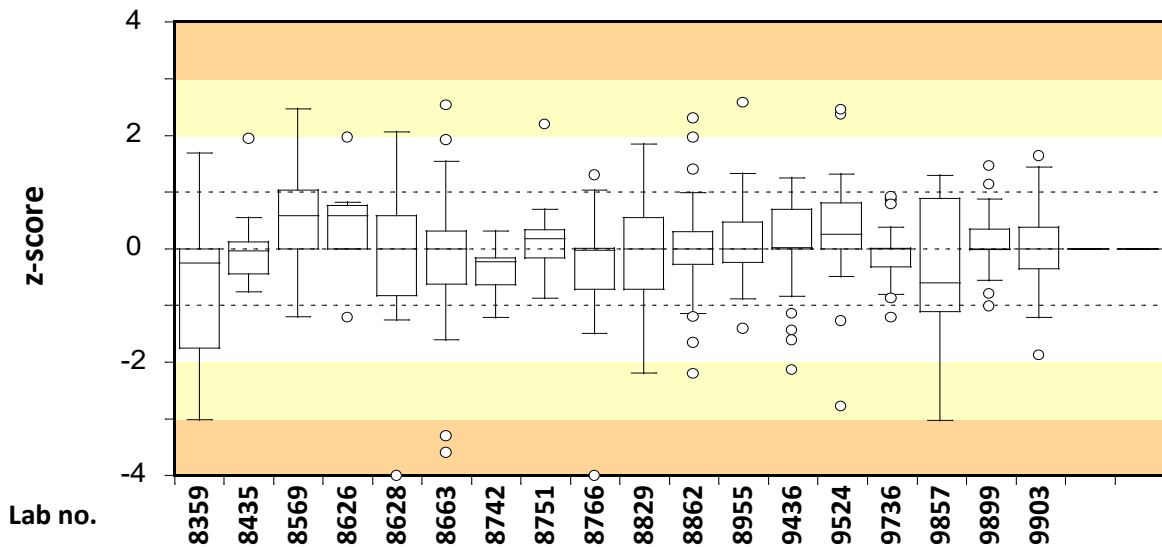
No. of results	12	20	0	11	32	18	23	29	29	20	12	14	12	0	6	0	23	29	9	3
False positive	0	2	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0
False negative	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Low outliers	0	1	0	0	0	0	0	0	0	3	2	3	0	0	0	0	0	0	0	0
High outliers	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0



No. of results	6	29	23	6	32	23	20	29	23	18	8	29	17	3	23	26	9	9	14	6
False positive	0	0	0	0	0	0	0	5	0	0	1	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	5	0	0	1	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	5	0	0	2	0	0	0	0	2	0	0	0	0
High outliers	0	0	0	0	0	0	0	2	1	0	1	0	1	0	3	2	0	0	0	0



No. of results	8	23	15	9	15	11	29	6	29	29	9	29	26	0	0	16	24	17	14	20
False positive	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
False negative	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Low outliers	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0



No. of results	14	12	23	9	23	20	9	11	29	18	32	32	29	18	23	12	29	23
False positive	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
False negative	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0
Low outliers	1	0	0	0	1	2	0	0	1	0	0	0	0	0	0	1	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Test material and quality control

Test material

Each participant received three samples with freeze-dried microorganisms, designated 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	15
	<i>Klebsiella pneumoniae</i>	SLV-537	-	-	50
	<i>Kluyveromyces marxianus</i>	SLV-439	-	CBS G99-106	60
	<i>Phialophora malorum</i>	SLV-545	Water	-	110
	<i>Macrococcus caseolyticus</i>	SLV-462	-	CCUG 35411	30*
B	<i>Enterobacter aerogenes</i>	SLV-099	-	-	1130
	<i>Clostridium perfringens</i>	SLV-442	-	CCUG 43593	30
	<i>Pichia kudriavzevii</i>	SLV-498	Human sputum	CCUG 35869	5
	<i>Cladosporium cladosporioides</i>	SLV-488	-	CBS 812.96	10
	<i>Aeromonas caviae</i>	SLV-081	Drinking water	CCUG 45103	2300
C	<i>Escherichia coli</i>	SLV-532	Water	CCUG 48891	35
	<i>Citrobacter freundii</i>	SLV-091	Water	CCUG 43597	20
	<i>Clostridium bifermentans</i>	SLV-009	Fish	CCUG 43592	6600
	<i>Sphingomonas species</i>	SLV-547	Water	-	60*
	<i>Streptomyces species</i>	SLV-548	Reservoir	-	140
	<i>Staphylococcus saprophyticus</i>	SLV-013	-	CCUG 45100	20*

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	A ¹			B ¹			C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Coliform bacteria (MF) SS-EN ISO 9308-1:2014	66	0.24	1.13	11 ²	1.19	1.91	54	2.25	1.47
Suspected thermotolerant colif. bact. (MF) SS 028167 (1996)	64	0.28	1.14	–	–	–	30	2.22	1.76
Escherichia coli (MF) EN ISO 9308-1:2014	15	0.17	1.23	–	–	–	36	1.90	1.57
Presumptive Clostridium perfringens (MF) SS-EN ISO 14189:2016	–	–	–	26	1.19	1.51	66 ²	2.60	1.45
Moulds (MF) SS 028192 (1989)	11 ³	0.52	1.56	25 ⁴	1.49	1.62	–	–	–
Yeasts (MF) SS 028192 (1989)	6 ³	0.56	1.97	8 ⁴	1.41	2.26	–	–	–
Actinomycetes (MF) SS 028212 (1994)	–	–	–	–	–	–	14 ³	1.55	1.84
Culturable microorg., 72 h 22 °C (pour plate) SS-EN ISO 6222:1999	33	2.24	1.66	38	1.16	1.39	18	0.93	1.53
Slow-growing bacteria, 7d 22 °C (pour plate) SS-EN ISO 6222:1999, modified	37	0.85	1.37	39	0.80	1.32	82	0.61	1.19

– No target organism or no value

¹ n = 5 vials analysed in duplicate

² cfu per 1 ml of sample

³ cfu per 10 ml of sample

⁴ cfu per 200 ml of sample

References

1. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption
3. Standard Methods for the Examination of Water and Wastewater, www.standardmethods.org
4. Ilbäck J and Blom L. 2024. Protocol – Microbiological Proficiency Testing, Swedish Food Agency.
5. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
6. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockfeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
7. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.

Annex 1. Results of the participating laboratories

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotol. 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	
1131	2	3	1	-	-	-	-	-	-	-	-	-	-	-	59	1300	43	16	0	31	-	-	-	-	-	-	-	-	-	-	-	-	-	59	37	16				
1237	1	2	3	-	-	-	47	1400	11	79	1600	<2	8	<1	4	43	>200	35	14	<1	25	-	-	-	46	740	170	-	-	-	-	-	-	48	19	17				
1263	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
1326	2	3	1	-	-	-	50	-	32	-	-	-	9	0	32	-	-	-	-	-	-	-	-	0	33	0	-	-	-	-	-	-	-	47	28	20				
1545	2	3	1	54	1380	42	54	<10	42	54	0	33	11	0	33	62	1076	40	14	0	33	0	44	3500	0	44	<10	63	6	0	81	5	0	0	0	158	46	35	21	
1611	3	1	2	63	2300	31	63	1100	31	46	0	28	12	0	21	58	1046	42,8	14,8	0	35,9	-	-	-	-	-	-	-	-	-	-	-	-	-	50	25	17			
1753	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	60	1704	40	10	0	26	0	29	5100	-	-	-	60	4	0	75	9	0	0	0	191	36	36	15	
1868	1	3	2	56	1141	51	56	1141	51	-	-	-	18	0	34	87	1301	42	18	0	27	10	20	5050	-	-	-	78	5	0	77	3	0	0	0	129	31	37	18	
1966	3	2	1	-	-	-	64	1288	32	-	-	-	17	0	20	59	1208	37	10	0	32	0	21	5500	-	-	-	80	12	0	86	0	0	0	0	177	52	32	13	
1970	1	2	3	45	3000	1	45	900	1	45	3000	1	11	<1	1	-	-	-	-	-	-	<1	27	2100	<1	27	<1	66	4	<1	75	6	<1	-	-	15	18	15		
2001	2	3	1	-	-	-	66	3000	76	-	-	-	22	<1	57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	55	20	23			
2317	2	3	1	54	910	21	54	910	21	-	-	-	13	0	17	-	-	-	-	-	-	0	7	3800	0	7	3800	-	-	-	-	-	-	-	-	21	23	12		
2745	1	3	2	55	860	55	55	860	55	11	0	36	11	0	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	38	16			
3055	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
3076	1	3	2	39	2250	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	27	20		
3145	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
3162	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	64	1553	30	14	0	27	0	40	3300	-	-	-	65	4	0	70	3	0	0	0	124	50	30	22	
3305	1	3	2	62	2300	32	62	1200	32	-	-	-	10	<1	25	50	1600	37	7	<1	30	<1	43	4900	<1	43	<1	130	21	<1	200	<1	<1	<1	<1	110	47	34	17	
3415	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	78	640	45	14	0	25	-	-	-	-	27	0	0	-	-	-	-	-	-	-	-	-	-	-	
3587	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	23	16		
3730	1	3	2	49	2300	52	-	-	-	52	0	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38	39	12			
3883	2	1	3	66	3580	51	66	1060	51	-	-	-	12	<1	39	62	1060	58	12	<1	38	<1	28	4090	-	-	-	106	10	<1	98	<1	<1	<1	<1	149	46	35	17	
4015	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	69	1553	56	13	0	38	0	31	2200	-	-	-	73	1	0	61	2	0	0	0	149	53	44	17	
4288	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	32	26	14	
4339	2	1	3	64	1400	33	64	1100	33	64	0	15	15	<100	20	64	1600	46	15	0	32	0	25	7400	0	25	<100	71	14	0	82	2	0	0	0	160	56	23	18	
4343	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	75	1203	58	17	0	43	0	28	1	-	-	-	78	7	0	76	4	0	0	0	101	35	35	28	
4356	2	1	3	53	1600	48	53	1000	48	56	0	11	11	0	38	67	1300	79	16	0	50	0	28	120	0	25	0	-	-	-	-	-	-	-	-	41	33	16		
4418	1	3	2	41	1030	13	41	1030	13	37	0	10	5	0	13	5	350	0	0	0	0	0	51	0	8	10	0	8	60	0	40	68	690	18	-	-	57	10	40	
4723	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	76	1120	61	11	<1	50	<1	32	7090	-	-	-	37	50	<1	93	4	<1	<1	<1	118	47	31	23	
4889	3	2	1	-	-	-	63	1300	46	-	-	-	13	0	29	45	1000	33	11	0	30	-	-	-	0	57	0	-	-	-	-	-	-	-	-	40	31	16		
5201	2	1	3	-	-	-	74	-	0	-	-	-	23	1945	1,27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	2960	1,2	-	-	-
5204	3	2	1	49	2700	46	49	1080	46	-	-	-	20	<1	28	68	1986	62	11	<1	41	<1	29	3200	-	-	-	65	5	<1	85	6	<1	<1	<1	190	32	33	19	
5220	3	1	2	-	-	-	65	1400	126	-	-	-	15	0	26	-	-	-	-	-	-	0	12	0	-	-	-	70	14	0	50	0	0	-	-	-	35	14	18	
5333	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	24	22		
5352	2	1	3	-	-	-	57	1300	45	61	0	21	18	0	31	-	-	-	-	-	-	0	50	4500	-	-	-	38	11	0	76	0	0	0	0	109	36	100	21	
5447	1	3	2	-	-	-	50	560	46	-	-	-	12	0	29	-	-	-	-	-	-	0	34	8300	0	34	0	9	70	0	5	140	0	0	0	0	160	55	32	18
5553	3	1	2	-	-	-	65	1310	23	-	-	-	16	0	23	-	-	-	-	-	-	-	-	-	0	40	0	-	-	-	-	-	-	-	-	-	-	-	-	-
5701	1	2	3	-	-	-	59	1300	55	-	-	-	18	0	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	37	18		
5858	3	2	1	-	-	-	49	1365	38	-	-	-	11	0	30	-	-	-	-	-	-	0	47	3475	0	47	0	-	-	-	-	-	-	-	-	-	55	37	21	
5950	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	150	-	-	-
6175	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	41	>200	4	6	0	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	31	23		
6182	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	56	1347	40	10	0	26	0	28	205	-	-	-	91	8	0	113	2	0	0	0	257	50	39	18	
6233	3	1	2	59	1500	39	59	1500	39	-	-	-	9	0	28	63	1730	26	10	0	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	22	14		
6253	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	65	1100	52	15	0	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	38	15	
6456	3	2	1	-	-	-	44	1000	40	-	-	-	15	<1	22	70	1100	53	15	<1	41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	29	14		
6686	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	69,7	1445	42,9	12,4	<1	27,1	51	29	5400	-	-	-	-	-	-	-	-	-	-	-	80	10	18		
7248	2	3	1	57	1900	41	57	380	41	47	0	14	23	0	29	62	1203	32	14	0	20	0	24	4700	-	-	-	62	13	0	69	1	0	0	0	100	37	23	14	
7381	2	3	1	-	-	-	39	1020	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	35	0		
7442	3	1	2	101	3164	1341	68	1152	45	-	-	-	18	0	32	59	1160	72	1																					

Annex 1. Results of the participating laboratories

8359	2	3	1	-	-	-	38	624	37	-	-	-	9	<1	31	-	-	-	-	-	-	<1	27	<1	<1	27	<1	-	-	-	-	-	-	-	-	-	-	61	11	9	
8435	1	3	2	-	-	-	53	1100	68	53	0	14	10	0	26	-	-	-	-	-	-	-	-	-	14	28	0	-	-	-	-	-	-	-	-	-	-	-	38	33	20
8569	3	2	1	86	2020	49	86	2020	49	-	-	-	14	0	36	86	1900	29	16	0	23	0	32	904	0	32	904	-	-	-	-	-	-	-	-	-	-	-	50	40	18
8626	1	2	3	64	2800	40	64	2000	40	33	0	0	8	0	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	38	13	
8628	3	2	1	61	1190	29	9	0	29	10	0	6	9	0	19	-	-	-	-	-	-	0	27	46	0	27	0	140	19	0	60	0	0	-	-	-	-	50	45	21	
8663	1	3	2	57	2500	38	57	1800	8	55	0	37	29	0	5	63	1100	47	21	0	36	0	18	8	0	18	0	-	-	-	-	-	-	-	-	-	59	29	16		
8742	3	1	2	-	-	-	48	1100	32	-	-	-	8	<1	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	29	19		
8751	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	53	1091	48	16	0	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	31	28		
8766	3	2	1	50	1290	34	50	<1	34	52	1082	14	10	<1	17	70	1320	61	12	<1	37	<1	30	6700	-	-	-	91	18	<1	89	<1	<1	<1	<1	140	40	22	12		
8829	2	1	3	-	-	-	50	1150	35	-	-	-	10	0	21	-	-	-	-	-	-	-	-	-	-	-	0	10	0	112	4	0	-	-	-	27	48	20			
8862	1	2	3	84	4700	48	84	2000	48	-	-	-	18	0	33	50	1388	47	12	0	32	0	31	5600	0	31	0	118	6	0	36	3	0	0	0	114	31	33	22		
8955	1	2	3	-	-	-	56	720	39	58	0	20	14	0	20	61	1200	56	11	0	39	0	37	4400	0	37	0	82	8	0	60	4	0	0	0	170	42	25	30		
9436	1	2	3	69	636	47	69	527	47	59	855	30	16	<1	30	47	1718	45	16	<1	30	<1	30	5000	-	-	-	50	5	<1	46	4	<1	<1	<1	173	53	36	21		
9524	2	3	1	-	-	-	70	1400	40	-	-	-	12	0	30	67	988	66	23	0	57	-	-	-	0	42	0	-	-	-	-	-	-	-	-	51	27	8			
9736	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	62	1442	33	14	0	25	0	26	5619	-	-	-	101	8	0	103	2	0	0	0	176	43	28	13		
9857	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	36	1733	33	5	<1	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	52	24	23			
9899	2	1	3	57	1305	43	57	1305	43	-	-	-	11	0	25	68	1370	34	19	0	27	0	22	0	-	-	-	80	11	0	78	1	0	0	0	150	49	41	18		
9903	3	1	2	-	-	-	54	1100	57	-	-	-	8	<1	23	-	-	-	-	-	-	-	-	-	-	-	-	119	5	<1	95	1	<1	<1	<1	142	45	46	15		
N				30	30	30	51	50	52	19	20	20	50	51	51	44	43	45	44	45	45	41	41	41	26	27	27	34	35	35	34	35	35	27	27	27	68	69	69		
n				30	30	30	50	46	47	19	20	20	49	50	46	43	42	43	42	45	44	36	39	41	22	23	22	32	33	34	32	33	34	27	27	27	64	64	66		
m _{PT}				-	-	-	7,561	34,262	6,235	-	-	-	3,656	-	5,130	7,886	36,468	6,689	3,668	-	5,628	-	5,462	-	-	5,727	-	8,764	3,018	-	8,919	1,386	-	-	-	12,193	6,674	5,556	4,203		
s _{PT}				-	-	-	0,693	5,296	1,032	-	-	-	0,683	-	0,805	0,717	3,971	1,085	0,473	-	0,783	-	0,768	-	-	0,924	-	1,489	0,935	-	1,328	1,105	-	-	-	1,358	0,673	0,743	0,494		
u _{PT}				-	-	-	0,121	0,966	0,181	-	-	-	0,122	-	0,141	0,135	0,757	0,204	0,090	-	0,148	-	0,152	-	-	0,231	-	0,324	0,198	-	0,285	0,234	-	-	-	0,327	0,103	0,112	0,075		
CV (%)				-	-	-	9	15	17	-	-	-	19	-	16	9	11	16	13	-	14	-	14	-	-	16	-	17	31	-	15	80	-	-	-	11	10	13	12		
u _{rel,mPT} (%)				-	-	-	1,3	2,3	2,3	-	-	-	2,7	-	2,2	1,4	1,7	2,4	2,0	-	2,1	-	2,2	-	-	3,2	-	3,0	5,2	-	2,6	13,5	-	-	-	2,1	1,2	1,6	1,4		
F+				-	-	-	0	0	0	-	-	-	0	1	0	0	0	0	0	0	0	5	0	-	4	0	5	0	0	1	0	0	1	0	0	0	0	0	0	0	
F-				-	-	-	0	3	1	-	-	-	1	0	0	0	0	1	1	0	1	0	1	-	0	2	0	1	0	0	0	0	0	0	0	0	0	1	0	1	
<				-	-	-	1	0	3	-	-	-	0	0	4	1	1	1	1	0	0	0	1	-	0	1	0	1	0	0	1	0	0	0	0	0	0	2	3	1	
>				-	-	-	0	1	1	-	-	-	0	0	1	0	0	0	0	0	0	0	0	-	0	1	0	0	2	0	1	2	0	0	0	0	0	1	2	1	
Min				39	636	1	9	0	0	10	0	0	0	0	1	5	350	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	100	0	10	0		
Max				101	4 700	1 341	86	3 000	126	79	3 000	40	29	1 945	57	87	1 986	79	23	0	57	51	50	8 300	46	740	3 800	140	70	40	200	690	18	0	0	257	80	2 960	40		
Med				57	1 747	41	57	1 120	40	52	0	14	12	0	28	63	1 300	45	14	0	31	0	29	3 500	0	32	0	78	8	0	79	2	0	0	0	150	45	31	18		
m _{PT}				-	-	-	57	1 174	39	-	-	-	13	-	26	62	1 330	45	13	-	32	-	30	-	-	33	-	77	9	-	80	2	-	-	-	149	45	31	18		
Lower				39	636	1	30	337	9	10	0	0	2	-	7	32	602	11	5	-	10	-	9	0	-	8	-	18	0	-	24	0	-	-	-	65	21	11	7		
Upper				101	4700	1341	93	2 516	88	79	3 000	40	33	-	57	101	2 341	99	26	-	64	-	61	8 300	-	73	-	176	34	-	167	23	-	-	-	265	76	61	33		

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

- 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

Annex 1. Results of the participating laboratories

Slow-growing microorganisms (cfu/ml)			Lab no.
A	B	C	
60	37	88	1131
50	20	61	1237
-	-	-	1263
-	-	-	1326
47	37	77	1545
50	25	41	1611
36	38	83	1753
36	41	82	1868
50	33	59	1966
-	-	-	1970
3	2	36	2001
-	-	-	2317
47	26	84	2745
-	-	-	3055
37	31	66	3076
-	-	-	3145
50	31	85	3162
-	-	-	3305
-	-	-	3415
-	-	-	3587
41	40	74	3730
49	36	64	3883
54	40	60	4015
39	28	74	4288
61	18	93	4339
35	32	82	4343
-	-	-	4356
59	11	11	4418
47	29	67	4723
-	-	-	4889
-	-	-	5201
39	33	65	5204
-	-	-	5220
-	-	-	5333
200	100	70	5352
30	30	90	5447
-	-	-	5553
-	-	-	5701
-	-	-	5858
40	38	87	5950
-	-	-	6175
51	39	84	6182
-	-	-	6233
-	-	-	6253
-	-	-	6456
-	-	-	6686
35	23	63	7248
-	-	-	7381
53	32	77	7442
-	-	-	7688
-	-	-	7728
34	32	65	7876
-	-	-	7930
-	-	-	7946
-	-	-	7962
-	-	-	7968
-	-	-	8068
-	-	-	8165
48	35	82	8260
44	40	72	8329

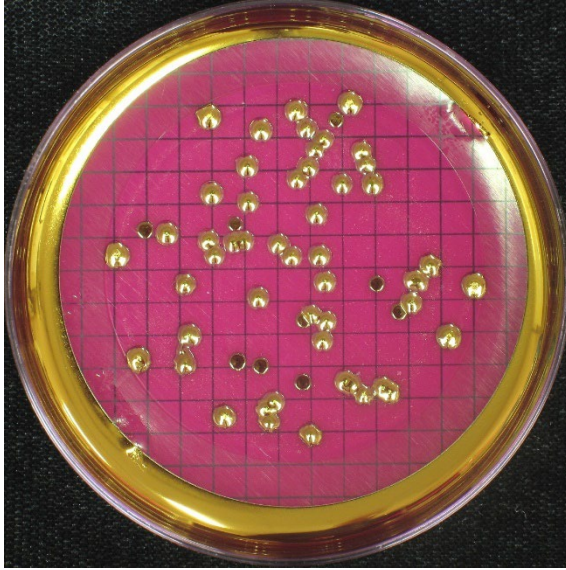
Annex 1. Results of the participating laboratories

-	-	-	8359
-	-	-	8435
53	41	79	8569
-	-	-	8626
62	36	69	8628
-	-	-	8663
-	-	-	8742
-	36	73	8751
43	25	24	8766
27	49	50	8829
34	36	65	8862
55	45	64	8955
54	37	87	9436
-	-	-	9524
43	30	70	9736
57	29	62	9857
52	41	82	9899
50	46	46	9903

40	41	41	N
38	38	39	n
6,771	5,796	8,388	m_{PT}
0,782	0,689	0,855	s_{PT}
0,155	0,134	0,167	u_{PT}
12	12	10	CV (%)
1,8	1,9	1,6	u_{rel,mPT} (%)
0	0	0	F+
0	0	0	F-
1	2	2	<
1	1	0	>
3	2	11	Min
200	100	93	Max
47	35	72	Med
46	34	70	m_{PT}
19	13	33	Lower
84	62	120	Upper

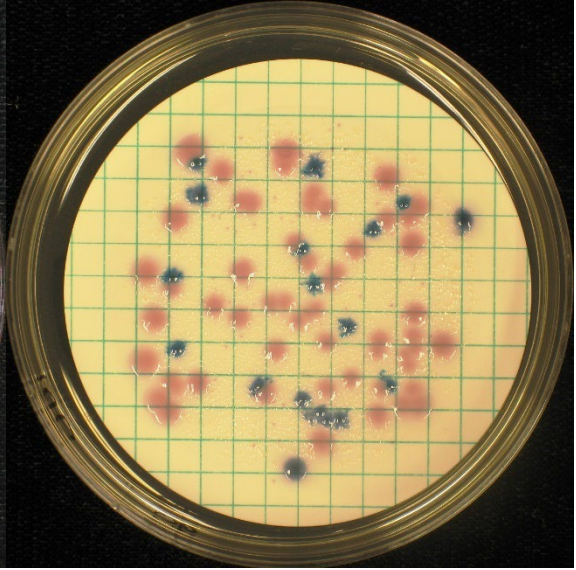
Sample A

m-Endo Agar LES, 37 °C



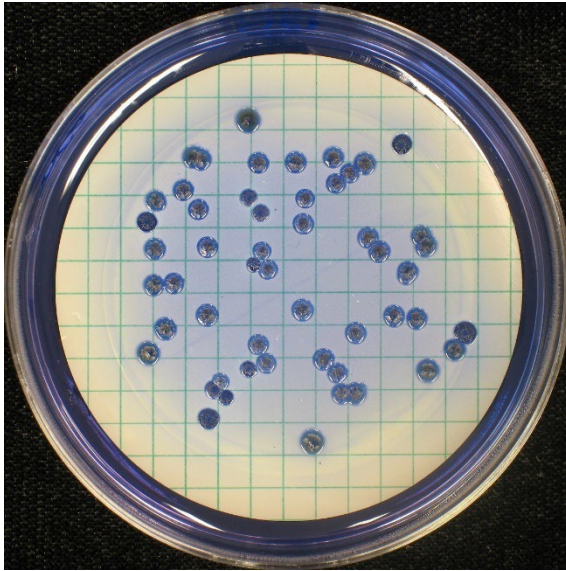
100 ml

Chromocult Coliform Agar, 37 °C



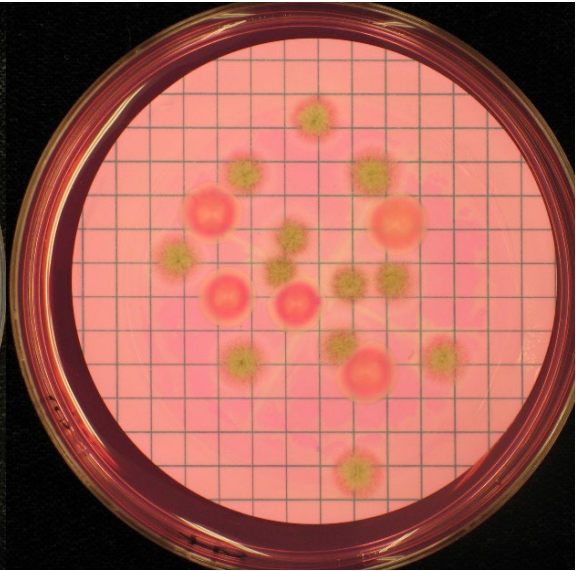
100 ml

m-FC Agar, 44 °C



100 ml

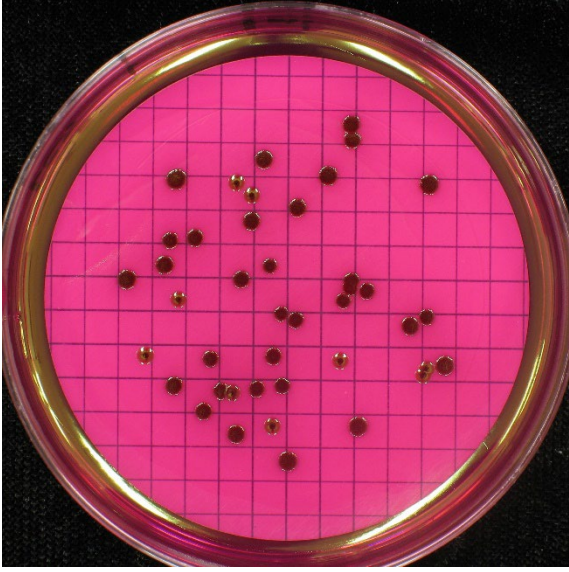
RBCC Agar, 25 °C



10 ml, 7 days

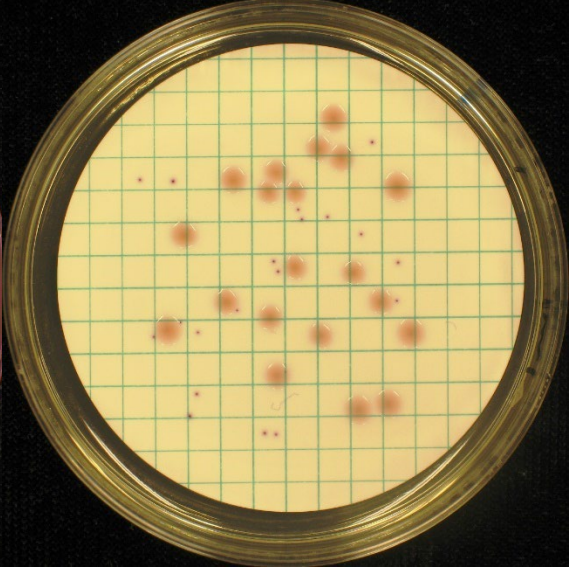
Sample B

m-Endo Agar LES, 37 °C



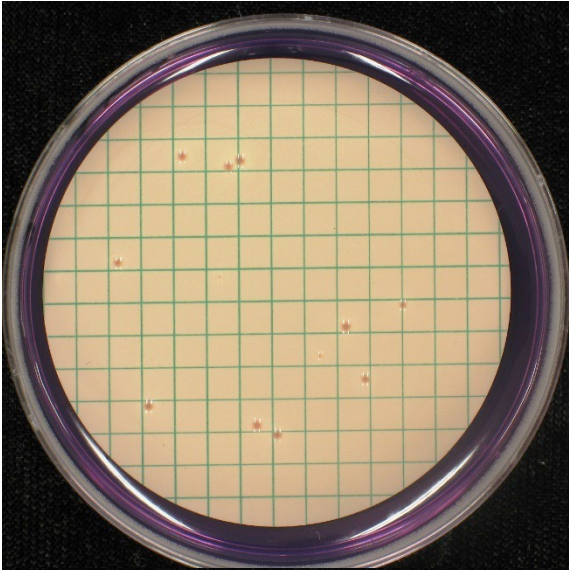
1 ml

Chromocult Coliform Agar, 37 °C



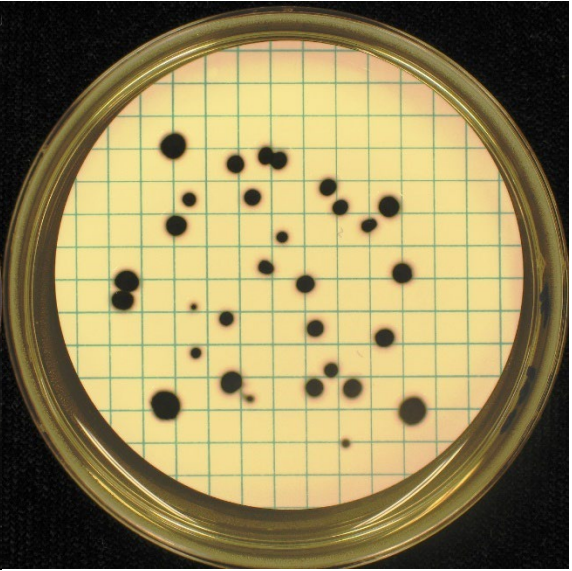
1 ml

m-FC Agar, 44 °C



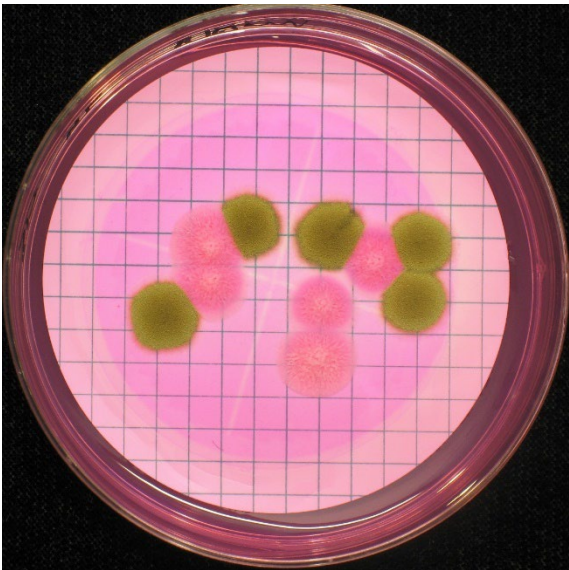
1 ml

TSC Agar, 44 °C



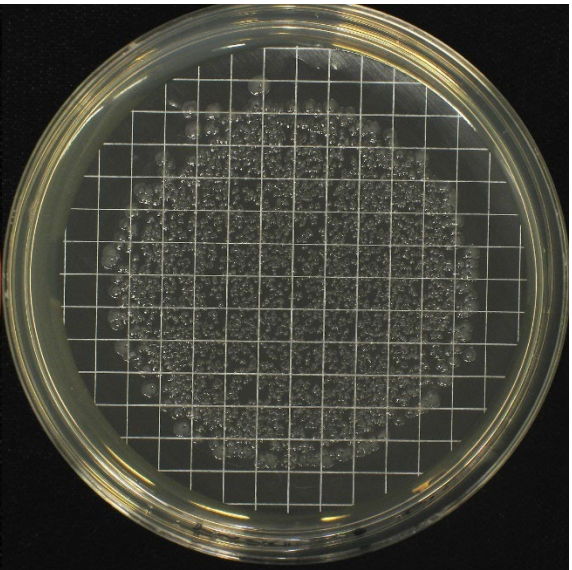
100 ml

RBCC Agar, 25 °C



100 ml

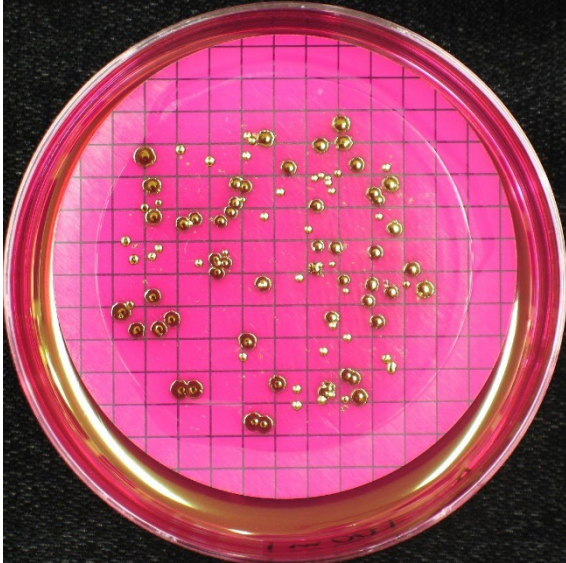
Actinomycete Isolation Agar, 25 °C



100 ml, 7 days

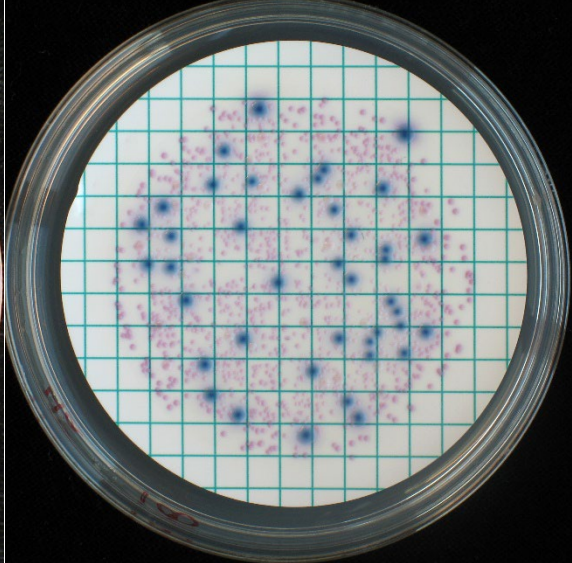
Sample C

m-Endo Agar LES, 37 °C



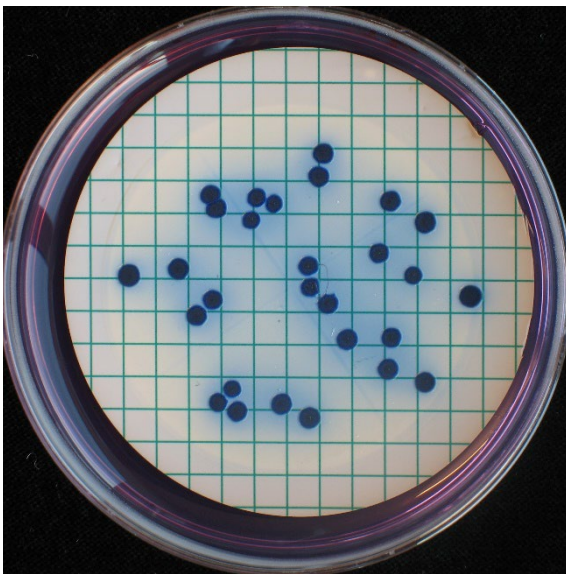
100 ml

Chromocult Coliform Agar, 37 °C



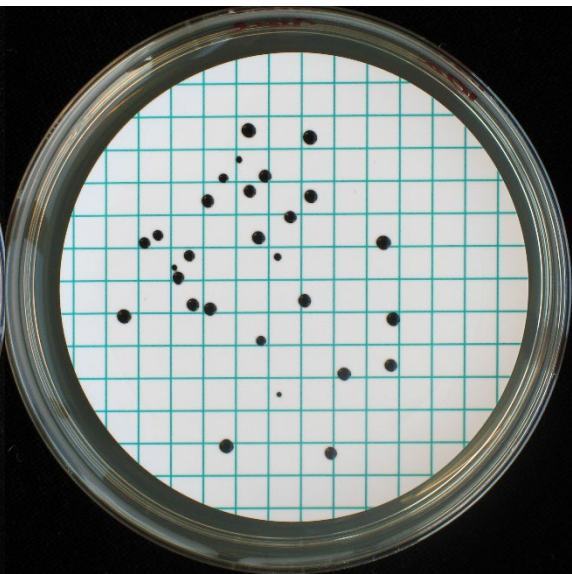
100 ml

m-FC Agar, 44 °C



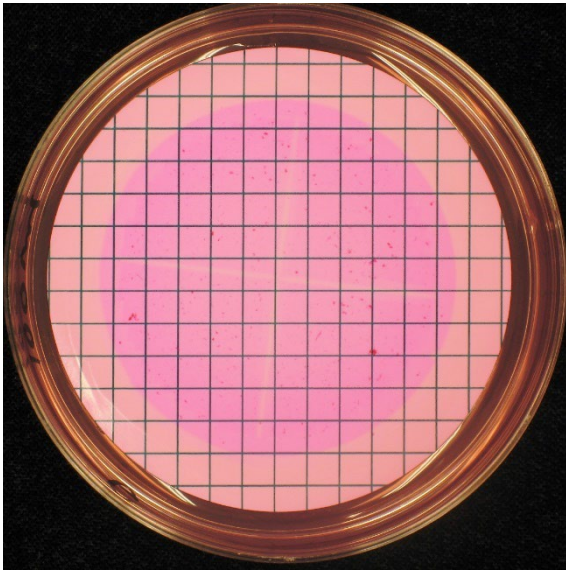
100 ml

TSC Agar, 44 °C



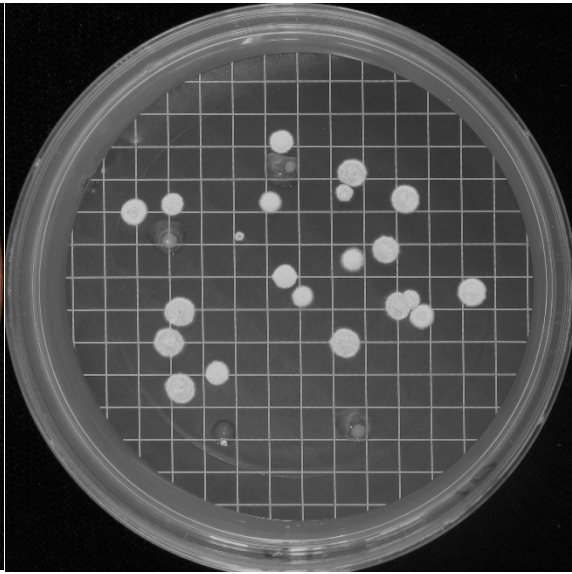
1 ml

RBCC Agar, 25 °C



100 ml, 7 days

Actinomycete Isolation Agar, 25 °C



10 ml, 7 days

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.

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