

Proficiency testing Drinking water Microbiology

September 2023

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Abbreviations

Media

BEAA	Bile Esculin Azide Agar (EN ISO 7899-2:2000)
CCA	Chromogenic Coliform Agar (EN ISO 9308-1:2014)
Colilert	Colilert® Quanti-Tray® (IDEXX Inc.; EN ISO 9308-2:2014)
Enterolert	Enterolert® Quanti-Tray® (IDEXX Inc.)
LES	m-Endo Agar LES (SS 028167)
LTLNB	Lactose tryptone lauryl sulphate broth (SS 028167)
m-Ent	m-Enterococcus Agar (EN ISO 7899-2:2000)
m-FC	m-FC Agar (SS 028167)
PACN	Pseudomonas Agar base/CN agar (EN ISO 16266:2008)
PCA	Plate count agar
Pseudalert	Pseudalert® Quanti-Tray® (IDEXX Inc.; ISO 16266-2:2018)
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

Intestinal enterococci

Pseudomonas aeruginosa

Culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours

Culturable microorganisms, 36 ± 2 °C for 44 ± 4 hours

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/Unknown”, 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 / MPN 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 86 participants; 36 in Sweden, 48 in other European countries, and two outside of Europe. Of the 78 participants that reported results, 29 (37 %) 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>Klebsiella oxytoca</i> <i>Enterococcus faecium</i> <i>Pseudomonas aeruginosa</i> <i>Stenotrophomonas maltophilia</i>				<i>Enterobacter cloacae</i> <i>Cronobacter sakazakii</i> <i>Enterococcus hirae</i> <i>Staphylococcus capitis</i>				<i>Escherichia coli</i> <i>Enterobacter cloacae</i> <i>Enterococcus durans</i> <i>Burkholderia cepacia</i> <i>Staphylococcus capitis</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. oxytoca</i>	51	0	4	<i>E. cloacae</i> <i>C. sakazakii</i>	52	0	3	<i>E. coli</i> <i>E. cloacae</i>	52	0	1
Susp. thermotol. coliform bact.	<i>E. coli</i>	19	-	-	(<i>E. cloacae</i>) (<i>C. sakazakii</i>)	19	-	-	<i>E. coli</i> (<i>E. cloacae</i>)	19	-	-
<i>E. coli</i> (MF)	<i>E. coli</i>	53	1	4	(<i>E. cloacae</i>) (<i>C. sakazakii</i>)	54	0	0	<i>E. coli</i>	54	0	0
Coliform bacteria (MPN)	<i>E. coli</i> <i>K. oxytoca</i>	49	0	3	<i>E. cloacae</i> <i>C. sakazakii</i>	51	0	2	<i>E. coli</i> <i>E. cloacae</i>	51	0	2
<i>E. coli</i> (MPN)	<i>E. coli</i>	50	0	3	-	51	0	0	<i>E. coli</i>	51	0	1
Intestinal enterococci	<i>E. faecium</i>	58	6	1	<i>E. hirae</i>	59	3	2	<i>E. durans</i>	59	0	3
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	47	0	4	-	48	0	0	(<i>B. cepacia</i>)	48	1	0
Culturable microorganisms, 22 °C for 3 days	<i>S. maltophilia</i>	72	0	2	<i>E. hirae</i> <i>E. cloacae</i> <i>C. sakazakii</i>	74	1	4	<i>E. cloacae</i> <i>B. cepacia</i> <i>E. durans</i> <i>E. coli</i>	74	0	4
Culturable microorganisms, 37 °C for 2 days	<i>S. maltophilia</i>	55	0	0	<i>S. capitis</i>	56	0	0	<i>S. capitis</i>	56	2	3

- 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. oxytoca* 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.

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

For most probable number (MPN) methods, 49 participants reported results. One high and two low outliers were reported.

Sample B

The strains of *C. sakazakii* and *E. cloacae* 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, 52 participants reported results for MF methods. One high and two low outlier were reported.

For MPN methods, 51 participants reported results. One high and one low outlier were reported.

Sample C

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

In total, 52 participants reported results for MF methods. One high outlier was reported.

For MPN methods, 51 participants reported results. One high and one low outlier were reported.

General remarks

For MF methods, most participants (49 %) 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.

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 on CCA and LES 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 followed EN ISO 9308-2:2012 using Colilert-18. Of these, 85 % used trays with 97 wells and 12 % used trays with 51 wells. 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 and Colilert-24 (Colilert) according to the manufacturers' instructions, and the group Other/Unknown in Table 3 used a multiple tube method based on lactose fermentation for the MPN method.

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	47	374	14	0	2	2	52	49	170	17	0	2	1	52	51	36	8	0	0	1
ISO 9308-1	25	23	373	17	0	2	0	25	23	159	19	0	2	0	25	25	37	9	0	0	0
SFS 3016	11	9	402	8	0	0	2	12	11	175	17	0	0	1	12	11	36	9	0	0	1
SS 028167	9	9	418	8	0	0	0	9	9	208	7	0	0	0	9	9	36	6	0	0	0
Other/Unknown*	6	6	315	10	0	0	0	6	6	164	15	0	0	0	6	6	36	9	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

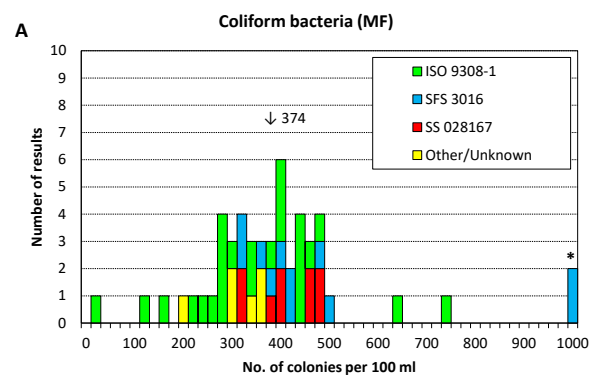
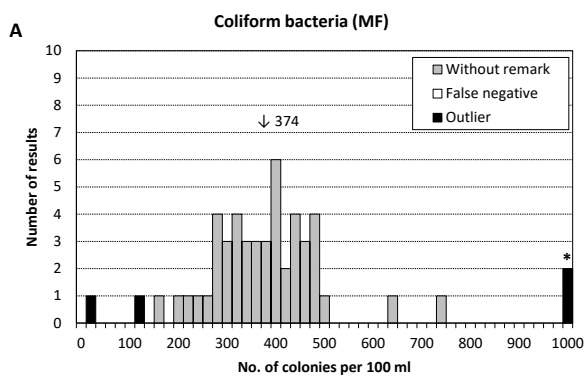
* The group Other/Unknown includes reporting of Swedish medical standard 2014.0, Bio-Rad RAPID'E. coli 2 agar, 3M™ Petrifilm™ Aqua Coliform Count Plates (6457/6458) and modified or withdrawn versions of SFS 3016 or ISO 9308-1.

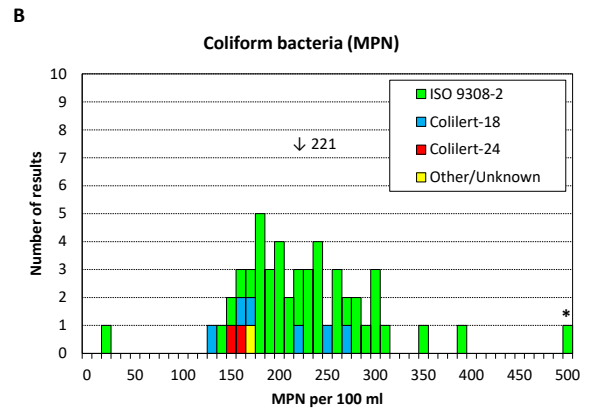
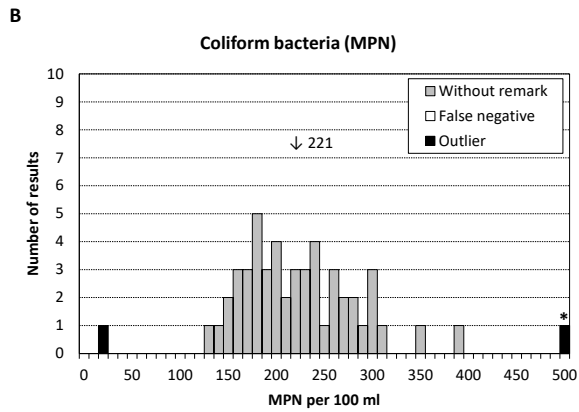
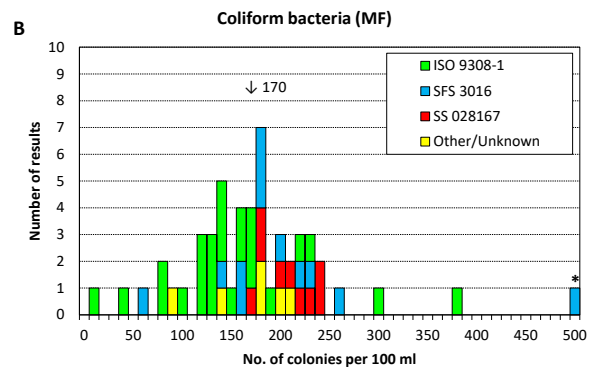
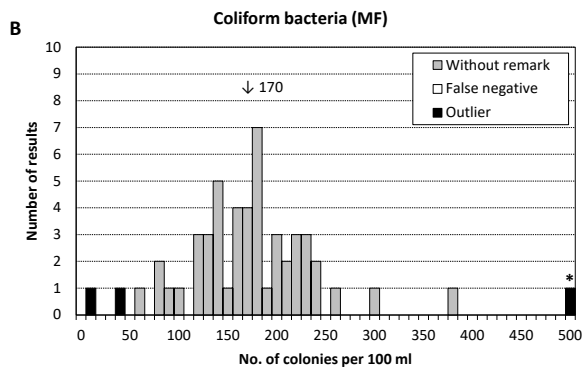
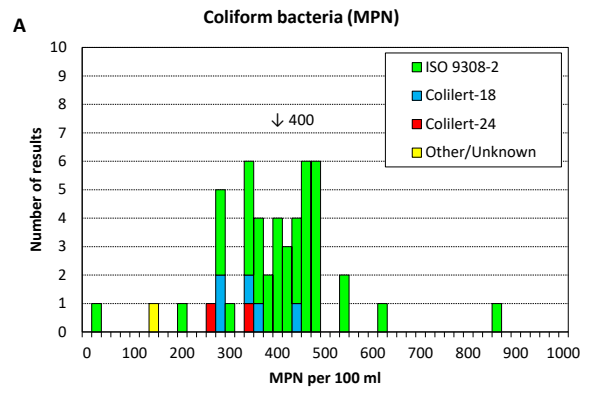
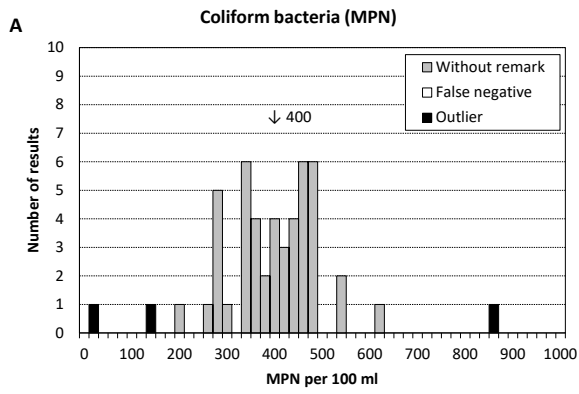
Table 3. Results from analysis of coliform bacteria with MPN methods.

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	49	46	400	12	0	2	1	51	49	221	14	0	1	1	51	49	39	10	0	1	1
ISO 9308-2	41	39	415	10	0	1	1	42	40	231	12	0	1	1	42	40	39	9	0	1	1
Colilert-18	5	5	349	9	0	0	0	6	6	199	14	0	0	0	6	6	39	11	0	0	0
Colilert-24	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
Other/Unknown	1	0	-	-	0	1	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 MPN scale

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





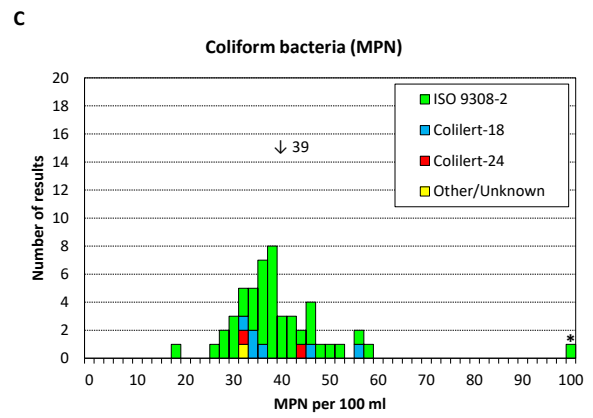
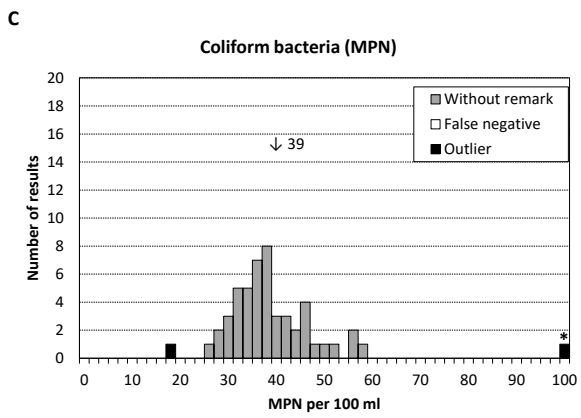
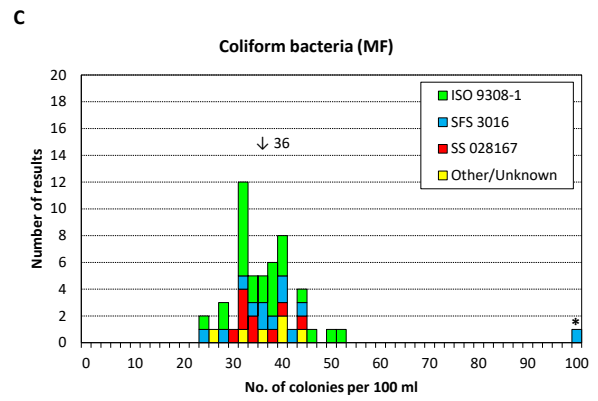
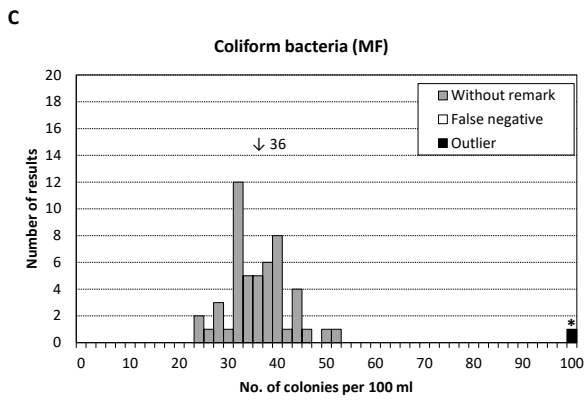


Figure 1. Results from analysis of coliform bacteria

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

The indole positive strain of *K. oxytoca* was present as a false-positive organism for the analysis.

In total, 53 participants reported results for MF methods. One false negative result, as well as two high and two low outliers were reported.

For MPN methods, 50 participants reported results. Three low outliers were reported.

Sample B

No target organism was present in the sample.

In total, 54 participants reported results for MF methods and 51 participants reported results for MPN methods. 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.

The concentration of *E. coli* was low; m_{PT} was only 6 cfu 100 ml⁻¹ and 5 MPN 100 ml⁻¹ and the z-scores should therefore be interpreted with caution. A reported result of <1 cfu 100 ml⁻¹ was included in the expected results range and is acceptable. Z-scores lower than -3 should be treated as acceptable.

In total, 54 participants reported results for MF methods.

For MPN methods, 51 participants reported results. One high outlier 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. EN ISO 9308 defines *E. coli* as a member of the Enterobacteriaceae that possesses both β -D-galactosidase and β -D-glucuronidase enzymes. On CCA, β -D-galactosidase and β -D-glucuronidase positive strains 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 EN 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	53	48	156	18	1	2	2	54	54	-	-	0	-	-	54	54	6	36	0	0	0
ISO 9308-1	28	27	156	14	0	1	0	28	28	-	-	0	-	-	28	28	5	31	0	0	0
SFS 3016, Modified	9	6	152	26	1	0	2	10	10	-	-	0	-	-	10	10	8	49	0	0	0
SS 028167, Modified	7	7	202	17	0	0	0	7	7	-	-	0	-	-	7	7	4	52	0	0	0
Other/Unknown	4	3	-	-	0	1	0	4	4	-	-	0	-	-	4	4	-	-	0	0	0
SS 028167	3	3	-	-	0	0	0	3	3	-	-	0	-	-	3	3	-	-	0	0	0
SFS 4088	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	2	-	-	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

* The group Other/Unknown include reporting of Swedish medical standard 2014.0, Schweizerisches Lebensmittelbuch (SLMB) 56; modified, Bio-Rad RAPID'E. coli 2 agar and a withdrawn version of ISO 9308-1.

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	50	47	166	12	0	3	0	51	51	-	-	0	-	-	51	50	5	29	0	0	1
ISO 9308-2	41	39	177	11	0	2	0	42	42	-	-	0	-	-	42	42	5	34	0	0	0
Colilert-18	6	6	161	6	0	0	0	6	6	-	-	0	-	-	6	5	5	25	0	0	1
Other/Unknown	2	1	-	-	0	1	0	2	2	-	-	0	-	-	2	2	-	-	0	0	0
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 MPN scale

* The group Other/Unknown include reporting of 4-methylumbelliferyl- β -D-glucuronide (MUG) with a tray with 96 wells and a multiple tube method based on lactose fermentation.

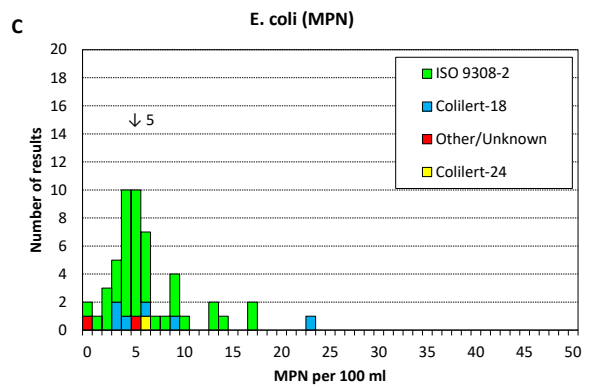
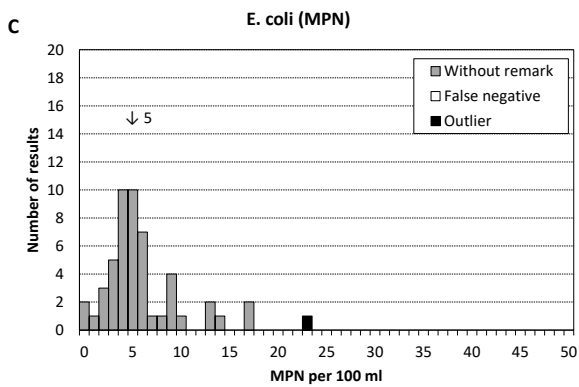
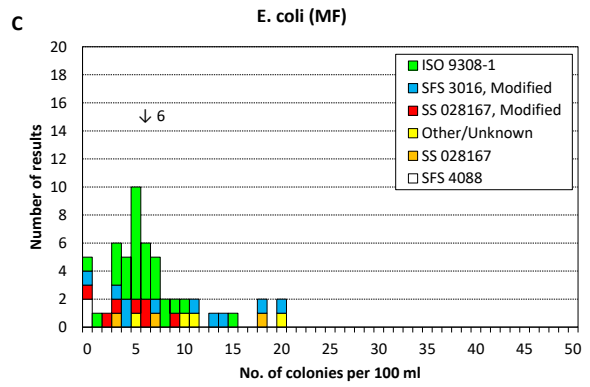
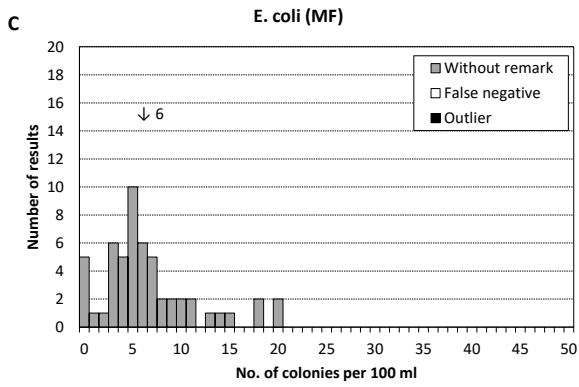
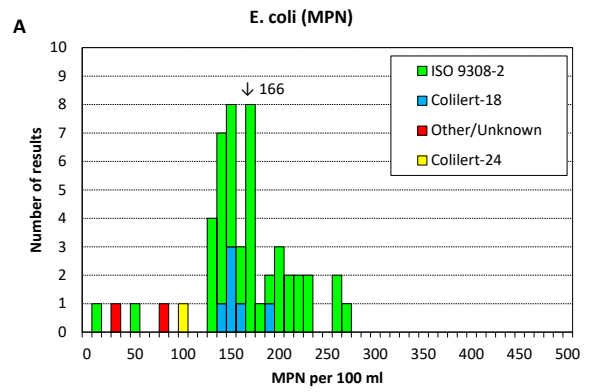
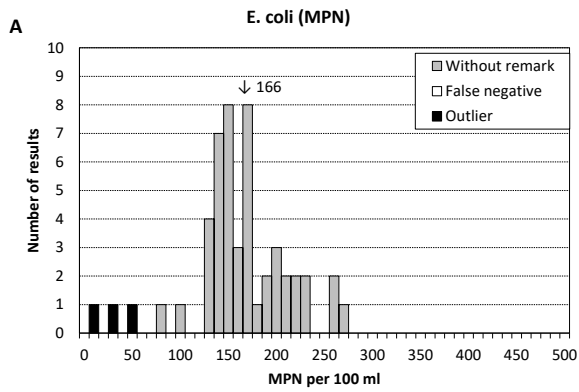
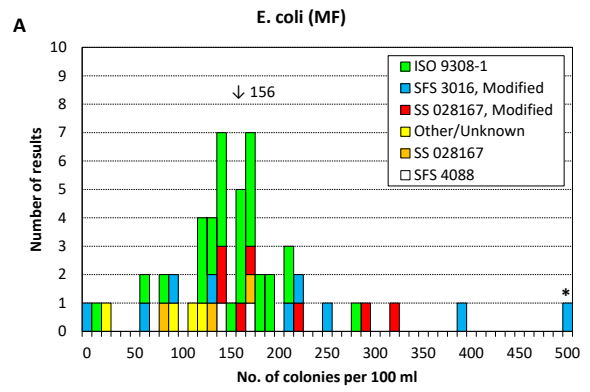
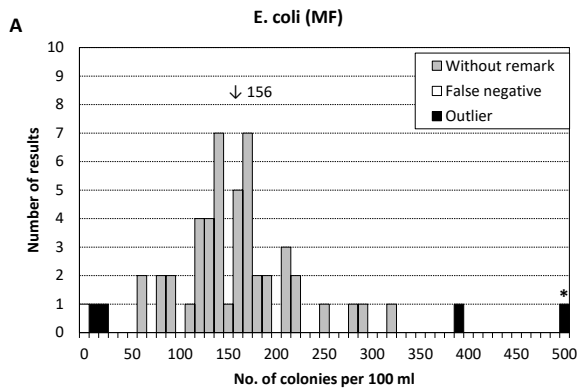


Figure 2. Results from analysis of *Escherichia coli*

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, 19 participants reported results.

Sample B

No thermotolerant coliform bacterium was present in the sample. However, on m-FC Agar, the strains of *E. cloacae* and *C. sakazakii* may grow as suspected thermotolerant coliform bacteria at 44/44.5 °C with blue or greyish colonies respectively.

In total, 19 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.

The strain of *E. cloacae* may form blue colonies on m-FC at 44/44.5 °C.

In total, 19 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, 19 participants reported results. Of these, 16 (84 %) 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	19	19	153	-	-	-	-	19	19	46	-	-	-	-	19	19	13	-	-	-	-
SFS 4088	7	7	123	-	-	-	-	7	7	30	-	-	-	-	7	7	10	-	-	-	-
SS 028167	6	6	152	-	-	-	-	6	6	74	-	-	-	-	6	6	14	-	-	-	-
Other/Unknown	4	4	-	-	-	-	-	4	4	-	-	-	-	-	4	4	-	-	-	-	-
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.

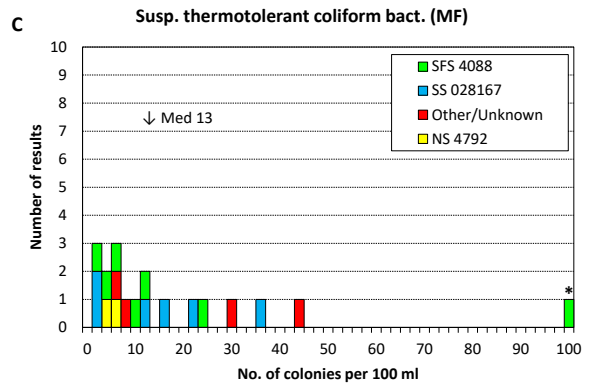
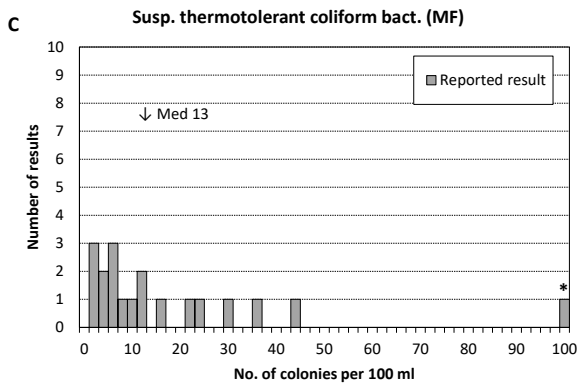
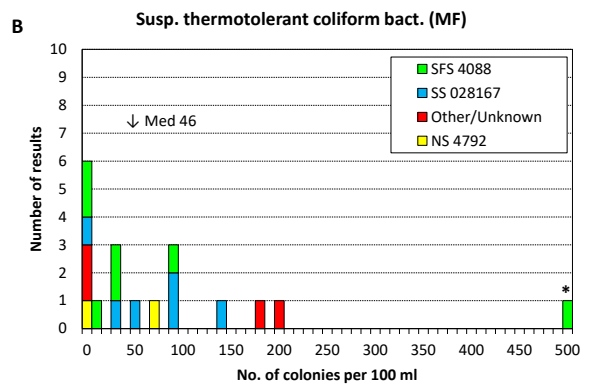
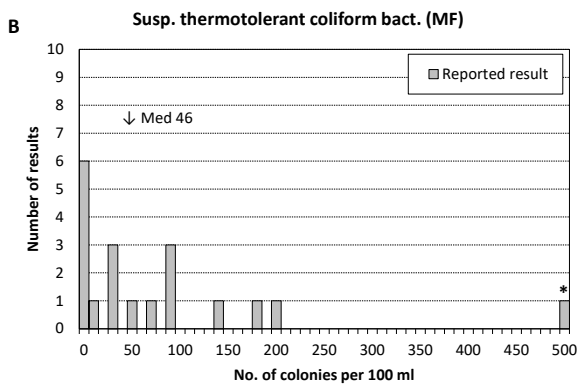
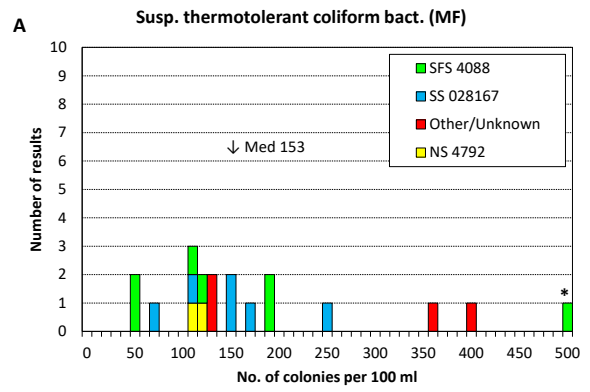
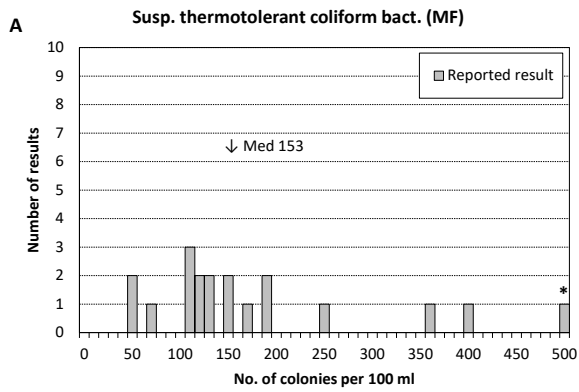


Figure 3. Results from analysis of *suspected thermotolerant coliform bacteria*

Intestinal enterococci

Sample A

The strain of *E. faecium* was target organism. On m-Enterococcus Agar (m-Ent), it forms light red to maroon colonies. Upon confirmation on Bile Esculin Azide Agar (BEAA), the blackening may be partially weak.

In total, 58 participants reported results. Six false negative results and one low outlier were reported.

The negative and low results may be due to interpretation of the confirmation. However, this strain has been found to have a low recovery on certain membrane filter batches. If this is experienced, the filters used should be compared with filter of another brand or batch.

Sample B

The strain of *E. hirae* was target organism. On m-Ent, it forms red colonies. Upon confirmation on BEAA, a distinct black colour is typically seen.

In total, 59 participants reported results. Three false negative result, as well as one high and one low outlier were reported.

Sample C

The strain of *E. durans* was target organism. On m-Ent, it forms maroon colonies. Upon confirmation on BEAA, a distinct black colour is typically seen.

The concentration of intestinal enterococci was low; m_{PT} was 6 cfu 100 ml⁻¹.

In total, 59 participants reported results. Three high outliers were reported.

General remarks

Most participants (86 %) followed EN ISO 7899-2:2000 using m-Ent (Slanetz & Bartley). According to the ISO standard intestinal enterococci are defined as bacteria that reduce 2,3,5-triphenyltetrazolium chloride to formazan and hydrolyse aesculin at 44 °C on m-Ent and BEAA, respectively.

Seven participants used Enterolert with the IDEXX Quanti-Tray System. Four of these used Enterolert-E and the other three used Enterolert-DW. ISO CD 7899-3 using Enterolert-DW is under development. The Enterolert-DW test defines intestinal enterococci as an organism that is capable of growth in the defined substrate medium, and that expresses the enzyme β -D-glucosidase producing a green colour through the enzymatic cleavage of ortho-nitrophenyl- β -D-glucoside.

The incubation temperature for m-Ent was 35, 36 or 37 °C. The incubation temperature was 41 °C for Enterolert, except for one participant that incubated at 37 °C.

Table 7. Results from analysis of intestinal enterococci.

Method	Sample A							Sample B							Sample C							
	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>	
All results	58	51	425	20	6	1	0	59	54	388	7	3	1	1	59	56	6	20	0	0	0	3
ISO 7899-2	50	44	415	21	6	0	0	51	47	397	6	2	1	1	51	48	6	19	0	0	0	3
Enterolert-E	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0	0
Enterolert-DW	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0	0
Other/Unknown*	1	0	-	-	0	1	0	1	0	-	-	1	0	0	1	1	-	-	0	0	0	0

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

For individual methods, *m*_{PT} = median value in cfu /MPN 100 ml⁻¹

* The group Other/Unknown include the reporting of SLMB 56, modified

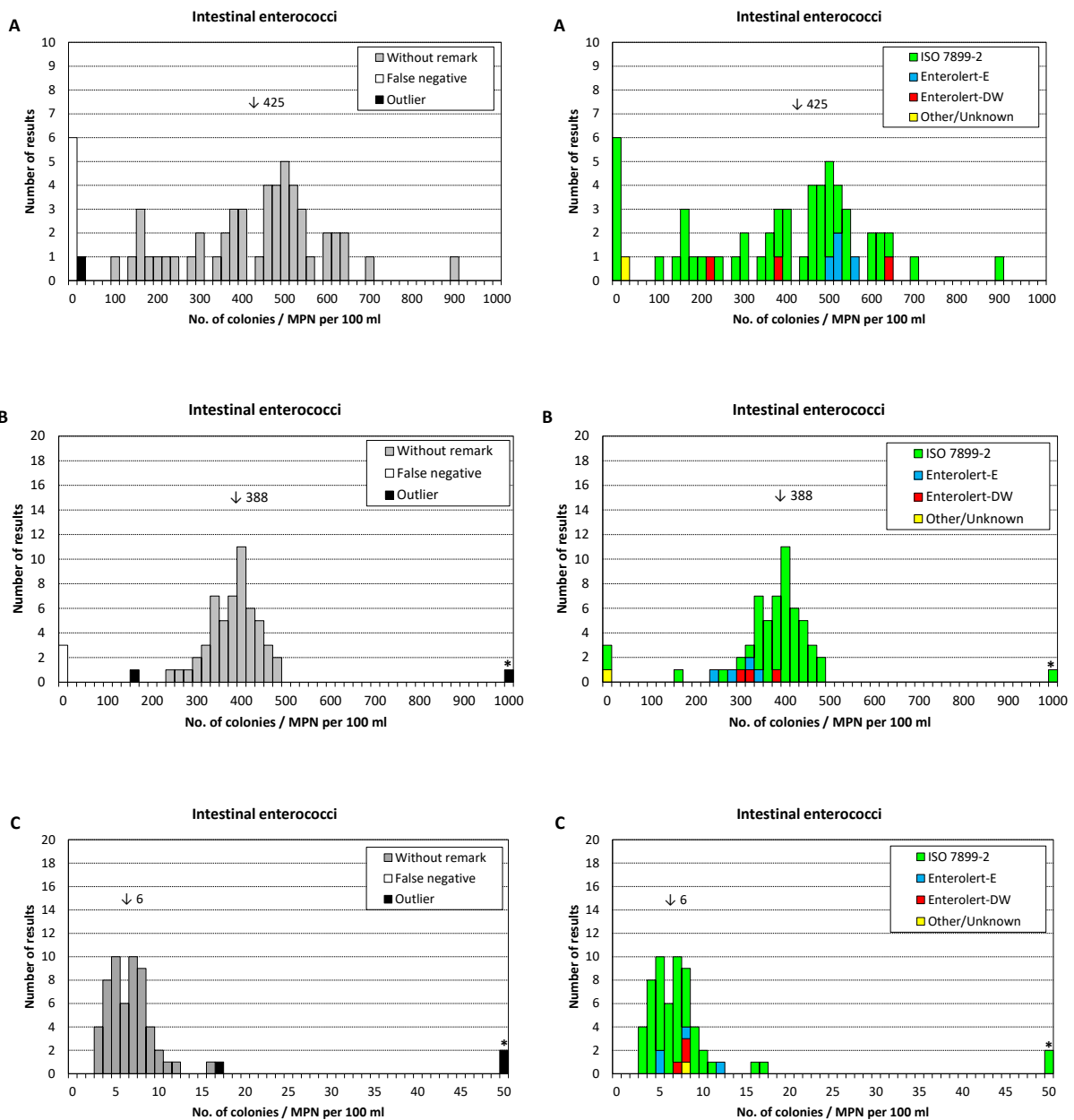


Figure 4. Results from analysis of intestinal enterococci.

Pseudomonas aeruginosa

Sample A

The strain of *P. aeruginosa* was target organism. On Pseudomonas Agar base/CN agar (PACN), it forms typical blue-green colonies that exhibit fluorescence under UV light.

In total, 47 participants reported results. Four high outliers were reported. Of these, three may have included the background flora as their result are similar to that concentration.

Sample B

No target organism was present in the sample.

In total, 48 participants reported results.

Sample C

No target organism was present in the sample. On PACN, *B. cepacia* may form transparent colonies.

In total, 48 participants reported results. One false positive result was reported.

General remarks

Most participants followed EN ISO 16266:2008. Since unhealthy substances like mercury are included, many laboratories have modified the standard and replaced the confirmation tests by another method. However, when only typical blue-green (pyocyanin-producing) colonies are present, no confirmation is required.

Seven participants followed ISO 16266-2 using Pseudalert and an additional four participants reported the use of Pseudalert according to the manufacturer's instructions.

Sample A included a typical pyocyanin-producing strain of *P. aeruginosa*. The results was rather dispersed with a *CV* of 25 %

Table 8. Results from analysis of *P. aeruginosa*.

Method	Sample A							Sample B							Sample C						
	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>	<i>N</i>	<i>n</i>	<i>m</i> _{PT}	<i>CV</i>	<i>F</i>	<	>
All results	47	43	176	25	0	0	4	48	48	-	-	0	-	-	48	47	-	-	1	-	-
ISO 16266	19	16	157	31	0	0	3	19	19	-	-	0	-	-	19	18	-	-	1	-	-
ISO 16266, Modified	13	12	162	26	0	0	1	14	14	-	-	0	-	-	14	14	-	-	0	-	-
ISO 16266-2	7	7	156	17	0	0	0	7	7	-	-	0	-	-	7	7	-	-	0	-	-
Pseudalert	4	4	-	-	0	0	0	4	4	-	-	0	-	-	4	4	-	-	0	-	-
Other/Unknown*	4	4	-	-	0	0	0	4	4	-	-	0	-	-	4	4	-	-	0	-	-

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

For individual methods, *m*_{PT} = median value in cfu / MPN 100 ml⁻¹

* The group Other/Unknown include the reporting of Compact Dry (Nissui Pharmaceutical), Pseudomonas Agar Bas without supplement, an internal method based on ISO 16266 and SLMB 56, modified.

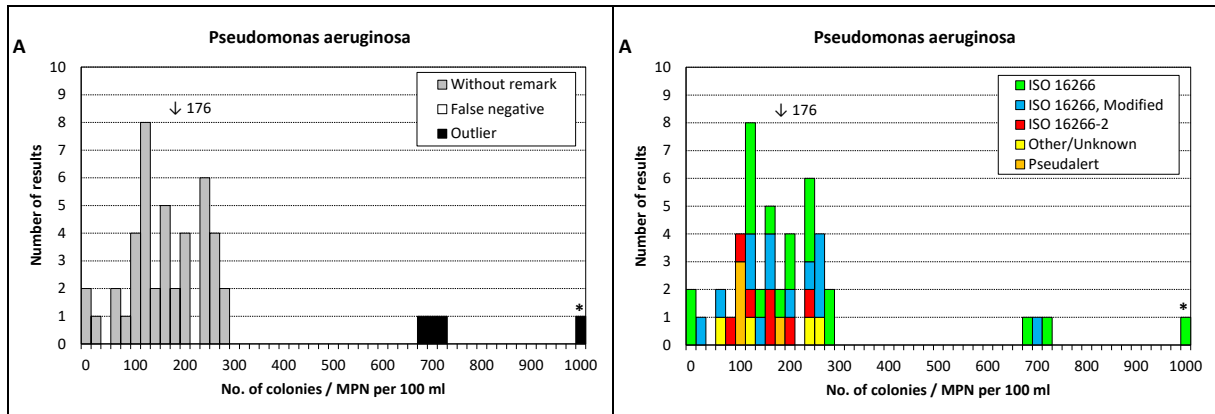


Figure 5. Results from analysis of *P. aeruginosa*.

Culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours

Sample A

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

In total, 72 participants reported results. One high and one low outlier were reported.

Sample B

The strains of *C. sakazakii*, *E. cloacae* and *E. hirae* were the main target organisms. The strain of *S. capitis* does not grow at 22 ± 2 °C.

In total, 74 participants reported results. One false negative result, as well as four high outliers were reported.

Sample C

The few colonies that might occur originate from *E. coli*, *B. cepacia*, *E. cloacae* and *E. durans*. The strain of *S. capitis* does not grow at 22 ± 2 °C.

The concentration of culturable microorganisms was low; m_{PT} was only 1 cfu 100 ml⁻¹. A reported result of <1 cfu 100 ml⁻¹ was included in the expected results range and is acceptable.

In total, 74 participants reported results. Four high outliers were reported.

General remarks

Seventy-one out of 74 participants followed EN ISO 6222:1999, which is based on a pour-plate method with Yeast extract Agar (YEA). Seven participants reported the use of Plate Count Agar (PCA) instead of YEA. Sixty-two participants reported to include both mould and yeasts in the result. Three participants included only yeasts and nine participants did not include either mould or yeast.

In Table 9 and Figure 6, the results are presented in relation to the magnification used for reading.

The high *CV* of 82 % in sample C is not statistically relevant, as it is mainly a consequence of the very low concentration of target organisms.

Table 9. Results from analysis of culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours.

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	72	70	28	13	0	1	1	74	69	7	21	1	0	4	74	70	1	82	0	0	4
ISO 6222	69	67	28	13	0	1	1	71	67	7	23	1	0	3	71	68	1	80	0	0	3
Other/Unknown*	3	3	-	-	0	0	0	3	2	-	-	0	0	1	3	2	-	-	0	0	1

Magnification	Sample A							Sample B							Sample C						
	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>
None	15	15	26	14	0	0	0	16	15	6	19	0	0	1	16	14	2	56	0	0	2
1.1-1.9x	18	17	29	14	0	0	1	19	18	8	17	0	0	1	19	19	1	80	0	0	0
2-4.9x	12	12	25	8	0	0	0	12	12	6	27	0	0	0	12	12	1	80	0	0	0
5-7.9x	4	4	-	-	0	0	0	4	3	-	-	0	0	1	4	4	-	-	0	0	0
8-11.9x	22	21	30	11	0	1	0	22	21	7	27	0	0	1	22	20	1	89	0	0	2
12-15.9x	1	1	-	-	0	0	0	1	0	-	-	1	0	0	1	1	-	-	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 group Other/Unknown includes reporting of Standard Methods, 9215 B [2], EasyDisc (IDEEX) and 3M™ Petrifilm™ Aerobic Count Plate.

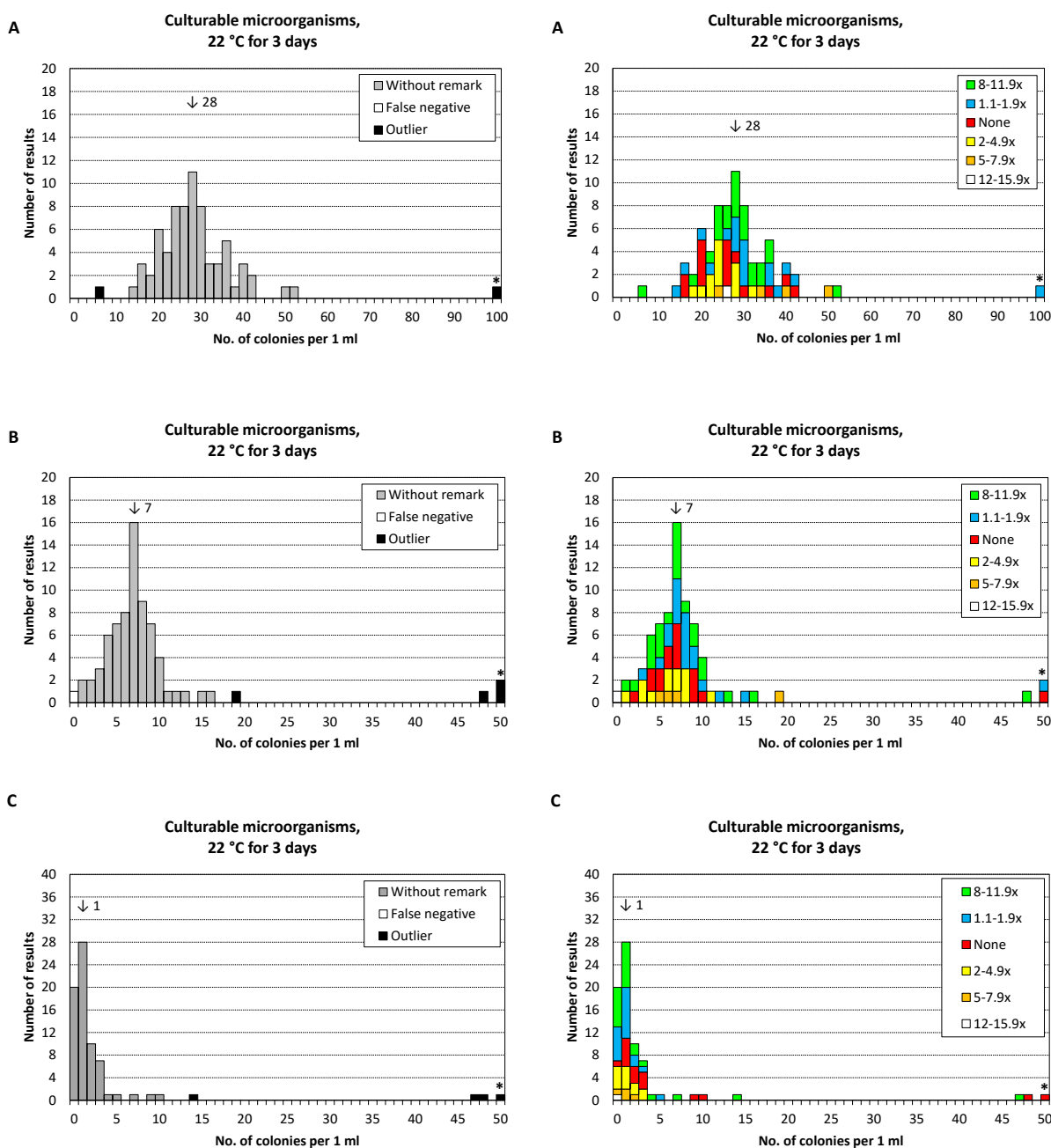


Figure 6. Results from analysis of culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours.

Culturable microorganisms, 36 ± 2 °C for 44 ± 4 hours

Sample A

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

In total, 55 participants reported results.

Sample B

The strain of *S. capitis* was the main target organism. The strains of *C. sakazakii*, *E. cloacae* and *E. hirae* also grow as culturable microorganisms, but in low numbers.

In total, 56 participants reported results.

Sample C

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

In total, 56 participants reported results. Two false negative results and three low outliers were reported.

The false negative results may be due to participants mixing up the reporting with culturable microorganisms, since the same participants have high outliers for culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours.

General remarks

Almost all participants (96 %) followed EN ISO 6222:1999. Of these, five reported the use of PCA instead of YEA. In Table 10 and Figure 7, the results are presented in relation to magnification for reading. Sample B and C both included the strain *S. capitis* that grows at 36 ± 2 °C but not at 22 ± 2 °C, as previously seen with this strain there are several low results that are inexplicable.

Table 10. Results from analysis of culturable microorganisms, 36 ± 2 °C for 44 ± 4 hours.

Method	Sample A							Sample B							Sample C						
	N	n	<i>m</i> _{PT}	CV	F	<	>	N	n	<i>m</i> _{PT}	CV	F	<	>	N	n	<i>m</i> _{PT}	CV	F	<	>
All results	55	55	26	12	0	0	0	56	56	32	22	0	0	0	56	51	39	22	2	3	0
ISO 6222	53	53	26	12	0	0	0	54	54	32	26	0	0	0	54	50	40	20	1	3	0
Other/Unknown*	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	1	-	-	1	0	0

Magnification	Sample A							Sample B							Sample C						
	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>	N	n	m _{PT}	CV	F	<	>
None	16	16	24	11	0	0	0	17	17	25	30	0	0	0	17	14	39	22	1	2	0
1.1-1.9x	13	13	26	11	0	0	0	13	13	33	29	0	0	0	13	12	42	19	0	1	0
2-4.9x	8	8	24	14	0	0	0	8	8	33	25	0	0	0	8	8	34	22	0	0	0
5-7.9x	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
8-11.9x	15	15	28	11	0	0	0	15	15	33	23	0	0	0	15	14	42	18	1	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 group Other/Unknown include reporting of Standard Methods [2] and the use of PCA (method unknown).

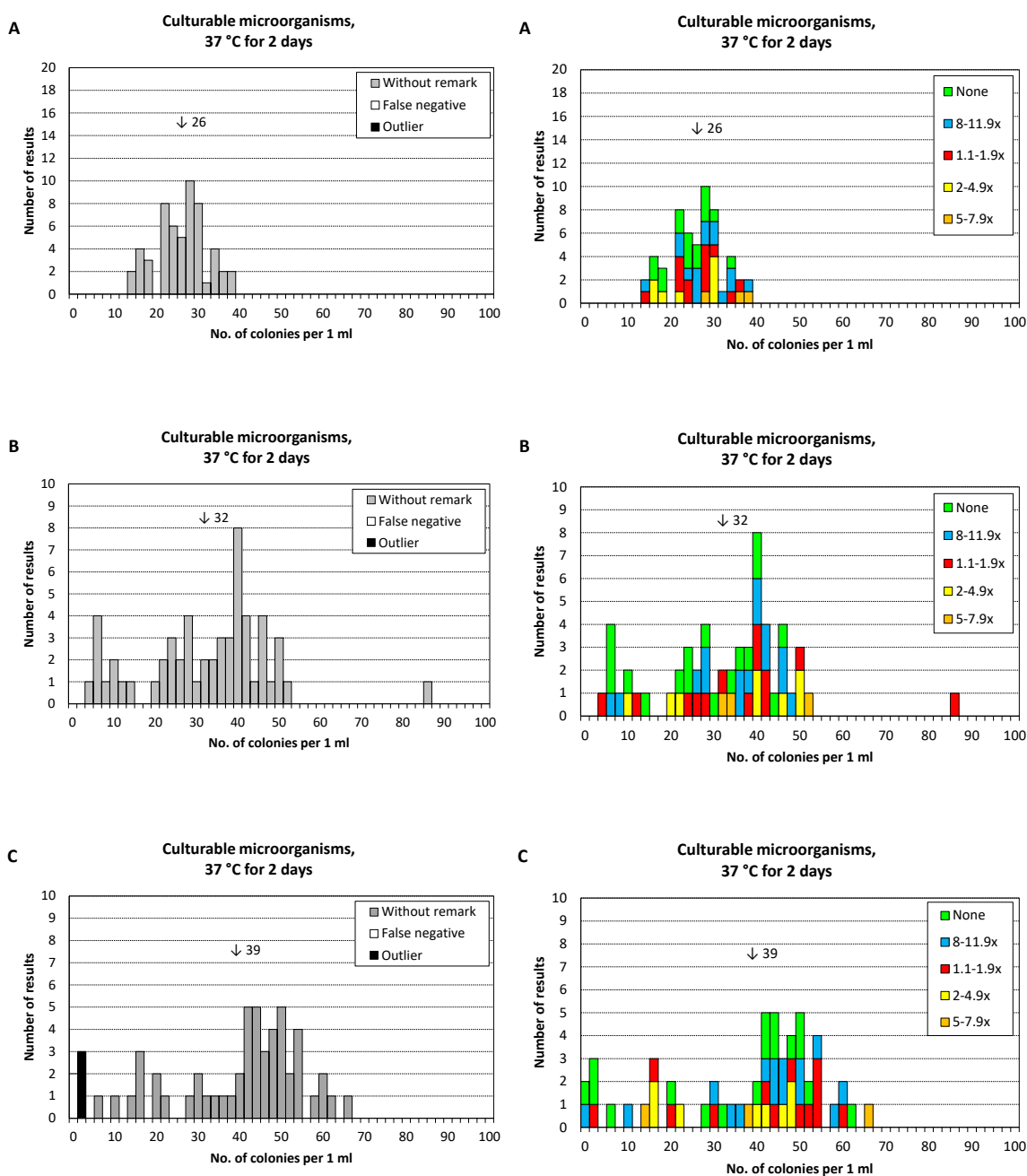


Figure 7. Results from analysis of culturable microorganisms, 36 ± 2 °C for 44 ± 4 hours.

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

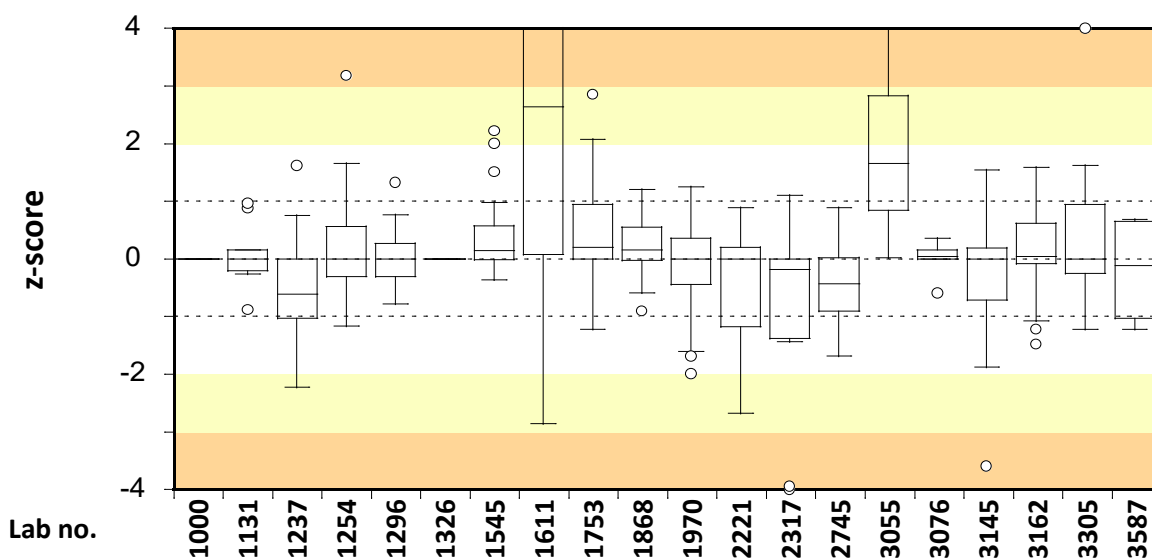
Box plots are based on the z -scores listed in Annex 2, and give a comprehensive view of the performance of each participant. The range of z -scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred around 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”.

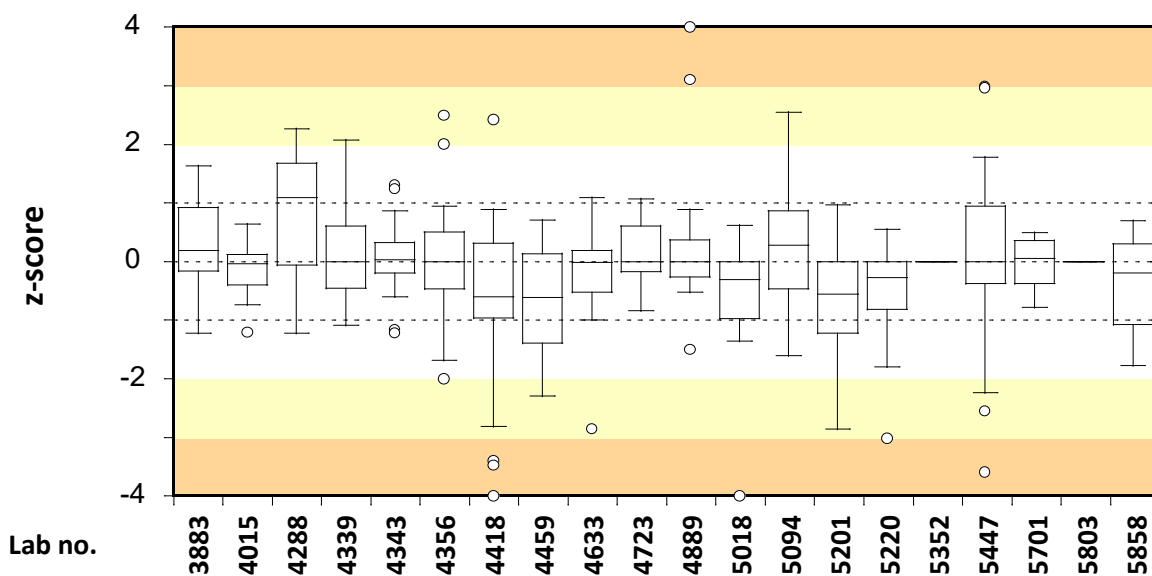
The participant’s median value is illustrated by a horizontal line in the box. Each box includes 50 % of a participant’s results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box. A circle is for technical 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.

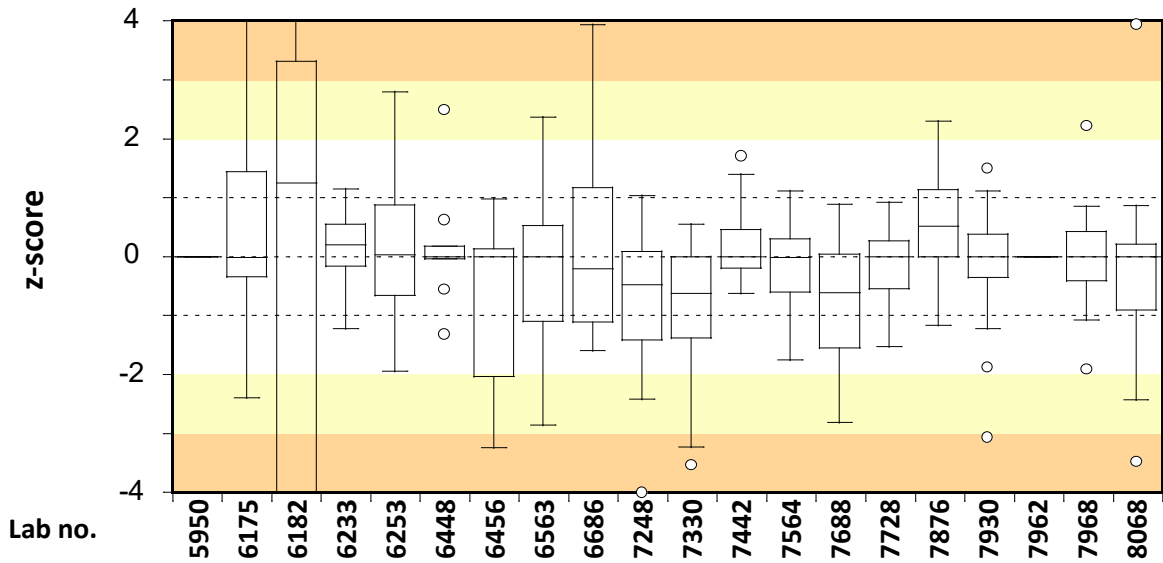
* $< [\text{lowest value in the box} - 1.5 \times (\text{highest value in the box} - \text{lowest value in the box})]$
or
 $> [\text{highest value in the box} + 1.5 \times (\text{highest value in the box} - \text{lowest value in the box})]$.



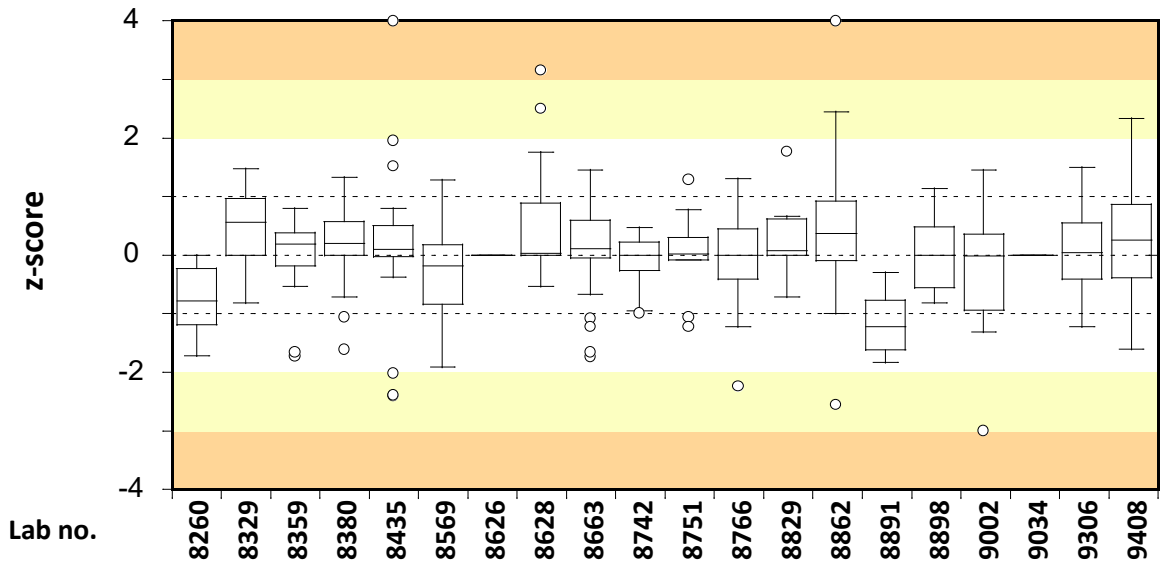
No. of results	0	9	24	24	18	0	24	24	18	18	18	12	18	9	3	9	18	18	24	6
False positive	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
False negative	0	0	1	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	1	0
Low outliers	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0
High outliers	0	0	0	1	0	0	0	10	0	0	0	0	0	0	1	0	0	0	1	0



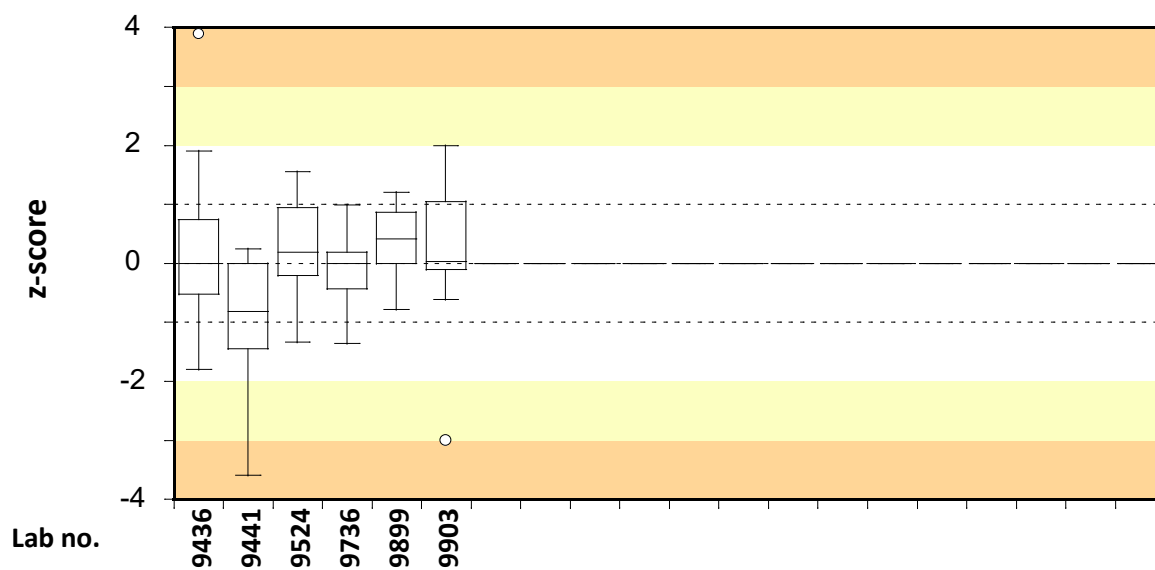
No. of results	24	12	3	24	18	24	24	15	21	12	24	12	15	6	24	0	18	6	0	18
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	2	0	0	0	0	1	0	0	1	0	1	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0



No. of results	0	11	12	20	12	9	18	24	12	24	12	18	12	24	18	24	24	0	24	24
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	3	0	0	0	1	0	0	1	2	0	0	0	0	0	1	0	0	1
High outliers	0	2	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1



No. of results	9	18	18	16	18	18	0	18	24	12	9	24	9	24	6	24	12	0	12	24
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0



No. of results	24	12	21	18	24	15
False positive	0	0	0	0	0	0
False negative	0	0	0	0	0	1
Low outliers	0	1	0	0	0	0
High outliers	1	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 11. Microorganisms and approximate concentrations in the samples.

Sample	Microorganism	Strain			cfu/100 ml ³
		SLV no. ¹	Origin	Reference ²	
A	<i>Escherichia coli</i>	SLV-165	Water	CCUG 43600	180
	<i>Klebsiella oxytoca</i>	SLV-089	Water	CCUG 43602	250
	<i>Enterococcus faecium</i>	SLV-459	-	CCUG 35172	540
	<i>Pseudomonas aeruginosa</i>	SLV-453	-	CCUG 551	330
	<i>Stenotrophomonas maltophilia</i>	SLV-041	-	CCUG 46537	20*
B	<i>Enterobacter cloacae</i>	SLV-451	-	CCUG 30205	170
	<i>Cronobacter sakazakii</i>	SLV-419	Water	-	35
	<i>Enterococcus hirae</i>	SLV-536	Water	CCUG 46536	395
	<i>Staphylococcus capitis</i>	SLV-463	-	CCUG 35173	40*
C	<i>Escherichia coli</i>	SLV-532	Water	CCUG 48891	6
	<i>Enterobacter cloacae</i>	SLV-451	-	CCUG 30205	35
	<i>Enterococcus durans</i>	SLV-078	Meat	CCUG 44816	6
	<i>Burkholderia cepacia</i>	SLV-042	-	-	20
	<i>Staphylococcus capitis</i>	SLV-463	-	CCUG 35173	50*

¹ Internal strain identification no. at the Swedish Food Agency

² Culture collection: 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 12. 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 ²			C ¹		
	m	I_2	T	m	I_2	T	m	I_2	T
Coliform bacteria (MF) SS-EN ISO 9308-1:2014	43 ³	0.96	1.34	23 ³	0.10	1.14	42	0.46	1.23
Suspected thermotolerant colif. bact. (MF) m-FC Agar, 44 °C according to SS 028167	12 ³	1.61	2.08	46	1.46	1.41	5	1.28	3.06
<i>Escherichia coli</i> (MF) SS-EN ISO 9308-1:2014	18 ³	1.18	1.71	-	-	-	6	0.36	1.62
Intestinal enterococci (MF) SS-EN ISO 7899-2:2000	54 ³	0.48	1.21	40 ³	0.56	1.27	6	1.86	3.04
<i>Pseudomonas aeruginosa</i> (MF) SS-EN ISO 16288:2008	33 ³	0.33	1.22	-	-	-	-	-	-
Culturable microorg., 48 h 37 °C (pour plate) SS-EN ISO 6222:1999	34	0.06	1.09	43	0.64	1.27	55	0.11	1.09
Culturable microorg., 72 h 22 °C (pour plate) SS-EN ISO 6222:1999	32	1.14	1.45	7	0.85	1.97	<1	-	-

– 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

References

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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-Lockefeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
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.	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria (MPN)			E. coli (MPN)			Susp. intestinal enterococci			Intestinal enterococci			Susp. Pseudomonas aeruginosa			Pseudomonas aeruginosa			Culturable microorganisms, 22 °C for 3 days			Culturable microorganisms, 37 °C for 2 days								
	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						
1000	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
1131	3	2	1	-	-	-	-	-	-	-	-	488	205	37	172	0	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	10	1	-	-	-					
1237	2	1	3	-	-	-	220	130	25	-	-	64	0	5	290	170	32	140	0	3	-	-	0	480	5	34	0	0	26	4	1	27	35	53								
1254	3	2	1	-	-	-	490	220	39	-	-	390	0	4	370	210	52	140	0	3	-	-	350	400	6	150	0	0	31	4	1	29	44	51								
1296	1	3	2	-	-	-	390	140	40	-	-	170	<1	3	-	-	-	-	-	-	-	-	390	370	8	250	<1	<1	39	7	1	22	28	44								
1326	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
1545	1	2	3	390	230	37	390	230	35	170	140	23	170	0	18	414	199	37	172	0	13	520	370	11	480	370	11	270	0	0	270	0	0	28	6	1	30	37	50			
1611	1	3	2	28400	3400	1900	28400	3400	1900	6400	2000	340	4000	0	0	866,4	517,2	59,4	224,7	0	17,1	1000	20600	188	900	20600	188	-	-	118,4	0	0	130	95	3	28	86	42				
1753	2	3	1	-	-	-	-	-	-	-	-	-	-	-	450	236	46	260	0	5	540	464	16	445	464	16	191	0	0	34	7	0	27	47	61							
1868	1	2	3	486	167	36	486	167	36	-	-	-	186	0	6	423	209	32	195	0	9	760	391	8	655	391	8	-	-	-	-	-	24	8	2	-	-	-				
1970	2	1	3	360	180	44	360	180	44	360	180	44	110	<1	10	-	-	-	-	-	-	400	410	3	400	410	3	150	<1	<1	150	<1	<1	25	8	1	30	10	16			
2221	3	1	2	-	-	-	-	-	-	-	-	-	210	<1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	6	7						
2317	2	1	3	-	-	-	30	18	32	-	-	-	12	0	5	-	-	-	-	-	-	-	-	-	0	0	8	-	-	-	240	0	24	27	6	1	34	15	20			
2745	2	3	1	287	133	28	287	133	28	132	0	6	132	0	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17	7	3	-	-	-				
3055	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	7	60	-	-	-			
3076	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
3145	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
3162	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	345	225	51	138	0	4	-	-	-	649	308	7	-	-	-	111	0	0	37	7	3	24	11	2			
3305	2	3	1	1020	160	45	1020	160	45	-	-	-	<1	<1	14	460	170	44	200	<1	9	510	450	8	290	450	8	130	7	22	130	<1	<1	27	6	<1	22	39	63			
3587	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
3883	1	2	3	491	248	33	491	248	33	-	-	-	164	<1	5	488	236	32	193	<1	6	363	455	4	363	455	4	250	<1	20	250	<1	<1	36	10	<1	38	42	37			
4015	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	292	186	37	176	0	5	541	390	7	541	390	7	-	-	-	-	-	-	27	5	1	-	-	-			
4288	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	15	0	-	-	-	
4339	1	2	3	-	-	-	430	140	36	110	14	10	220	0	4	550	180	39	260	0	4	560	380	5	520	340	12	-	-	-	160	0	0	32	7	3	31	23	23			
4343	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	411	308	41	167	0	4	491	356	6	464	356	6	-	-	-	179	0	0	35	4	0	35	46	43			
4356	3	1	2	400	200	42	400	200	42	58	30	12	210	0	20	290	220	46	150	0	13	500	390	5	500	390	5	170	0	0	110	0	0	17	8	1	15	25	48			
4418	1	2	3	290	130	38	290	130	38	130	0	8	130	0	8	140	170	33	33	0	0	480	430	9	480	430	9	36	20	28	15	0	0	21	5	9	18	22	48			
4459	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	261	150	44	86	0	5	-	-	-	496	403	5	-	-	-	-	-	-	18	3	1	19	20	49			
4633	3	1	2	-	-	-	373	180	32	123	3	5	149	0	0	310	200	40	130	0	9	-	-	-	409	360	9	-	-	-	-	-	-	25	8	1	23	50	43			
4723	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	366	291	46	160	0	3	409	436	6	409	427	6	-	-	-	-	-	-	-	-	-	-	-	-	-		
4889	1	2	3	-	-	-	440	150	34	-	-	-	140	0	5	490	210	28	170	<1	5	-	-	-	530	360	17	-	-	-	1100	0	0	30	7	2	28	29	33			
5018	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	280	260	37	150	0	2	-	-	-	460	160	5	-	-	-	130	0	0	-	-	-	-	-	-	-		
5094	3	2	1	-	-	-	317	127	53	-	-	-	129	0	9	-	-	-	-	-	-	-	-	-	474	443	3	-	-	-	-	-	-	-	-	-	25	7	2	29	53	67
5201	3	2	1	-	-	-	-	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
5220	2	1	3	-	-	-	130	123	33	-	-	-	83	0	4	390	184	39	158	0	4	-	-	-	231	397	8	-	-	-	116	0	0	17	6	2	16	31	43			
5352	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
5447	2	1	3	-	-	-	740	380	47	-	-	-	150	0	15	-	-	-	-	-	-	-	-	-	240	440	7	-	-	-	200	0	0	27	2	1	24	6	2			
5701	1	2	3	400	200	40	-	-	-	-	-	-	135	0	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29	8	2	-	-	-	
5803	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
5858	3	1	2	-	-	-	256	100	39	-	-	-	128	0	7	-	-	-	-	-	-	-	-	535	343	4	175	343	4	-	-	-	160	0	0	23	9	0	25	43	50	
5950	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
6175	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
6182	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	>200	130	36	165	0	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	70	48	26	7	0	
6233	3	2	1	280	-	-	280	-	-	-	-	-	210	-	-	387	240	43	172	0	9	520	420	6	520	420	6	91	0	0	91	0	0	30	8	0	23	39	61			
6253	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	480	180	39	150	0	17	-	-	-	530	290	5	-	-	-	-	-	-	-	-	-	21	10	10	-	-	-
6448	1	2	3	-	-	-	370	180	40	-	-	-	90	0	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
6456	1	2	3	-	-	-	380	230	24	-	-	-	170	<1	<1	473	190	18	171	<1	4	-	-	-	501	255	5	-	-	-	-	-	-	-	-	15	7	1	-	-	-	
6563	3	1	2	400	200	30	400	200	30	400	200	30	320	0	0	461	289	56	156	0	6	560	330	8	560	330	8	61	0	0	61	0	0	19	4	0	26	36	35			
6686	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	450	164	56	150	<1	2																					

Annex 1. Results of the participating laboratories

Lab no.	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria (MPN)			E. coli (MPN)			Susp. intestinal enterococci			Intestinal enterococci			Susp. Pseudomonas aeruginosa			Pseudomonas aeruginosa			Culturable microorganisms, 22 °C for 3 days			Culturable microorganisms, 37 °C for 2 days							
	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					
7968	2	1	3	-	-	-	430	180	40	55	9	24	130	0	18	410	190	36	140	0	7	-	-	-	160	340	4	-	-	-	260	0	0	27	9	1	25	37	44		
8068	1	2	3	-	-	-	320	160	34	190	34	6	64	0	3	201	140	34	55	0	2	-	-	-	470	400	7	-	-	-	700	0	0	21	7	2	29	46	41		
8260	2	1	3	119	86	25	263	86	28	-	-	-	143	0	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21	6	1	-	-	-				
8329	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	548	308	43	231	0	6	615	345	8	490	345	8	-	-	-	206	0	0	28	9	4	33	41	55		
8359	3	1	2	-	-	-	414	86	33	-	-	-	141	0	5	-	-	-	-	-	-	-	-	0	405	7	-	-	-	205	0	0	31	3	2	30	42	54			
8380	1	3	2	-	-	-	-	180	40	-	-	-	0	11	-	245	31	-	0	10	-	-	-	-	350	3	-	-	-	0	0	-	9	2	-	40	43	-			
8435	1	3	2	-	-	-	170	300	39	116	5	6	170	0	7	-	-	-	-	-	-	-	-	-	150	270	7	-	-	-	720	0	0	28	12	1	24	40	54		
8569	1	3	2	326	173	32	326	173	32	-	-	-	140	0	3	432	263	38	173	0	3	630	460	4	160	460	4	-	-	-	-	-	36	4	0	-	-	-			
8626	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
8628	3	2	1	-	-	-	410	170	33	120	73	5	170	0	5	-	-	-	-	-	-	-	-	460	410	9	370	410	9	270	0	48	270	0	0	50	19	1	39	33	38
8663	1	2	3	500	260	36	500	260	36	190	90	2	250	0	7	440	260	43	140	0	5	530	410	4	180	400	4	240	0	0	240	0	0	28	3	0	31	41	47		
8742	3	1	2	-	-	-	280	120	39	-	-	-	140	<1	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31	7	1	25	29	46			
8751	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	429	271	31	222	0	5	-	-	-	-	-	-	-	-	-	-	-	29	7	0	-	-	-			
8766	3	1	2	336	220	41	336	220	41	115	97	36	140	<1	9	461	308	39	210	<1	5	560	387	4	318	387	4	160	<1	<1	160	<1	<1	25	2	<1	23	27	44		
8829	2	1	3	-	-	-	440	170	32	-	-	-	166	<1	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	9	1	-	-	-				
8862	1	2	3	455	227	33	455	227	33	-	-	-	190	0	5	630	396	41	205	0	4	618	336	5	618	336	5	245	0	0	245	0	0	31	48	47	30	6	0		
8891	1	2	3	-	-	-	300	90	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	<1	<1	-	-	-				
8898	2	1	3	464	180	39	464	180	39	-	-	-	227	0	6	345	180	34	173	0	4	545	345	5	545	345	5	209	0	0	209	0	0	32	9	2	22	28	31		
9002	1	2	3	-	-	-	310	200	36	-	-	-	90	0	13	-	-	-	-	-	-	-	-	-	-	630	390	7	-	-	-	-	24	1	0	-	-	-			
9034	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
9306	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	291	227	34	158	0	3	-	-	-	-	-	-	-	-	-	-	-	30	8	1	37	50	53			
9408	1	3	2	-	-	-	649	140	50	-	-	-	285	0	8	350	272	46	147	<1	14	500	460	3	500	460	3	120	<2	<2	120	<2	<2	25	6	2	30	40	41		
9436	2	3	1	355	140	41	213	140	41	155	34	3	128	0	4	348	352	34	132	0	5	700	400	4	700	400	4	691	10	0	691	0	0	29	13	3	28	43	44		
9441	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	344	165	32	104	<1	6	-	-	-	-	-	-	-	-	-	-	20	5	1	28	5	2				
9524	1	2	3	-	-	-	460	230	36	-	-	-	160	<1	4	495	241	37	216	<1	4	-	-	-	-	-	-	-	-	-	-	41	6	5	35	27	20				
9736	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	364	231	29	140	0	4	555	367	7	500	367	7	168	0	0	168	0	0	29	5	0	29	48	47		
9899	1	3	2	448	197	40	448	197	40	-	-	-	171	0	3	489	281	48	200	0	6	610	434	10	610	434	10	135	0	30	135	0	0	31	7	1	26	41	59		
9903	2	1	3	-	-	-	473	241	44	256	98	13	172	0	6	-	-	-	-	-	-	-	-	-	0	382	5	-	-	-	282	0	0	26	1	7	-	-	-		
N				24	23	23	51	52	52	19	19	19	53	54	54	49	51	51	50	51	51	35	35	35	58	59	59	22	22	22	47	48	48	72	74	74	55	56	56		
n*				-	-	-	50	52	51	-	-	-	52	-	54	49	51	51	50	-	51	-	-	-	52	55	59	-	-	-	47	-	-	72	73	74	55	56	54		
m _{PT}				-	-	-	19,334	13,019	6,011	-	-	-	12,477	-	2,388	19,991	14,860	6,207	12,874	-	2,286	-	-	-	20,605	19,690	2,547	-	-	-	13,269	-	-	5,310	2,635	1,002	5,133	5,681	6,264		
s _{PT}				-	-	-	2,632	2,185	0,499	-	-	-	2,285	-	0,855	2,397	2,054	0,607	1,569	-	0,658	-	-	-	4,157	1,366	0,508	-	-	-	3,342	-	-	0,705	0,546	0,826	0,632	1,265	1,349		
u _{PT}				-	-	-	0,465	0,379	0,087	-	-	-	0,396	-	0,145	0,428	0,360	0,106	0,277	-	0,115	-	-	-	0,721	0,230	0,083	-	-	-	0,609	-	-	0,104	0,080	0,120	0,106	0,211	0,229		
CV (%)				-	-	-	14	17	8	-	-	-	18	-	36	12	14	10	12	-	29	-	-	-	20	7	20	-	-	-	25	-	-	13	21	82	12	22	22		
u _{rel,mPT} (%)				-	-	-	1,9	2,3	1,2	-	-	-	2,5	-	4,9	1,7	1,9	1,4	1,7	-	4,0	-	-	-	2,8	0,9	2,6	-	-	-	3,7	-	-	1,6	2,4	9,6	1,7	3,0	2,9		
F+				-	-	-	0	0	0	-	-	-	0	0	0	0	0	0	0	0	0	-	-	-	0	0	0	-	-	-	0	0	1	0	0	0	0	0	0		
F-				-	-	-	0	0	0	-	-	-	1	0	0	0	0	0	0	0	0	-	-	-	6	3	0	-	-	-	0	0	0	0	1	0	0	0	0	2	
<				-	-	-	2	2	0	-	-	-	2	0	0	2	1	1	3	0	0	-	-	-	1	1	0	-	-	-	0	0	0	1	0	0	0	0	3		
>				-	-	-	2	1	1	-	-	-	2	0	0	1	1	1	0	0	1	-	-	-	0	1	3	-	-	-	4	0	0	1	4	4	0	0	0		
Min				119	40	25	30	18	24	55	0	2	0	0	0	25	24	18	13	0	0	250	0	3	0	0	3	15	0	0	15	0	0	6	0	0	15	5	0		
Max				28 400	3 400	1 900	28 400	3 400	1 900	6 400	2 000	340	4 000	0	20	866	517	454	272	0	24	5 066	20 600	690	900	20 600	690	691	20	48	1 100	0	24	130	95	60	39	86	67		
Med				400	180	36	390	173	36	132	34	10	160	0	5	412	220	38	167	0	5	540	0	6	474	398	6	169	0	0	160	0	0	28	7	1	27	36	44		
m _{PT}				-	-	-	374	170	36	-	-	-	156	-	6	400	221	39	166	-	5	-	-	-	425	388	6	-	-	-	176	-	-	28	7	1	26	32	39		
Lower				-	-	-	130	41	20	55	0	2	31	-	0	163	75	19	66	-	0	-	-	-	66	243	1	-	-	-	10	-	-	10	1	0	10	3	4		
Upper				-	-	-	742	384	57	6 400	2 000	340	374	-	25	739	442	65	310	-	19	-	-	-	1 094	566	17	-	-	-	543	-	-	56	19	13	50	90	107		

N = number of reported results
n = results without annotation

Min = lowest reported result
Max = highest reported result

Med = median value
m_{PT} = assigned value

s_{PT} = standard deviation
u_{PT} = measurement uncertainty

F+ = false positive
F- = false negative
< = low outlier
> = high outlier

Lower = lowest accepted value
Upper = highest accepted value

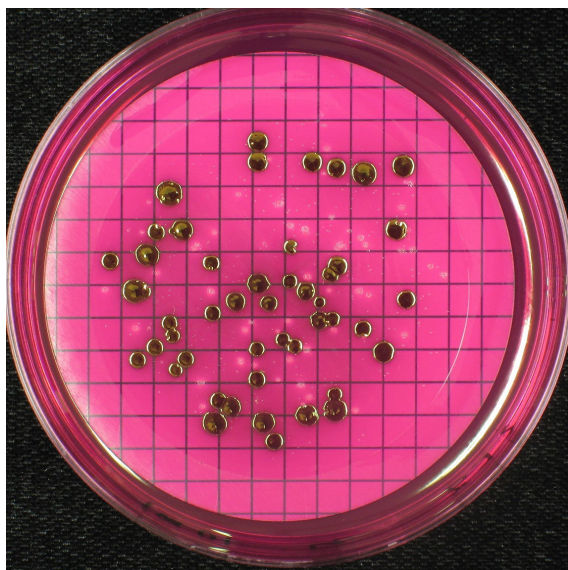
Annex 2. Z-scores of all participants

Lab no.	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria (MPN)			E. coli (MPN)			Susp. intestinal enterococci			Intestinal enterococci			Susp. Pseudomonas aeruginosa			Pseudomonas aeruginosa			Culturable microorganisms, 22 °C for 3 days			Culturable microorganisms, 37 °C for 2 days			
	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	
7968				0.533	0.182	0.630				-0.471	0	2.169	0.107	-0.524	-0.341	-0.664	0	0.547				-1.914	-0.916	-1.077				0.855	0	0	-0.161	0.669	-0.003	-0.211	0.318	0.274	
8068				-0.549	-0.169	-0.360				-1.959	0	-0.768	-2.425	-1.474	-0.619	-3.477	0	-1.325				0.259	0.227	0.193				3.947	0	0	-1.032	0.020	0.499	0.399	0.871	0.103	
8260				-1.184	-1.714	-1.442				-0.227	0	-0.768																									
8329													1.426	1.309	0.578	1.481	0	0.249																			
8359				0.385	-1.714	-0.533				-0.264	0	-0.178																									
8380					0.182	0.630								0.386	-1.053		0	1.333																			
8435				-2.392	1.968	0.470				0.245	0	0.301																									
8569				-0.486	0.061	-0.709				-0.282	0	-0.768	0.331	0.661	-0.070	0.178	0	-0.842																			
8626																																					
8628				0.347	0.009	-0.533				0.245	0	-0.178																									
8663				1.150	1.421	-0.021				1.459	0	0.301	0.411	0.615	0.578	-0.664	0	-0.076																			
8742				-0.988	-0.945	0.470				-0.282	0	0.071																									
8751													0.301	0.780	-1.053	1.291	0	-0.076																			
8766				-0.381	0.830	0.787				-0.282	0	0.716	0.617	1.309	0.063	1.031	0	-0.076																			
8829				0.624	0.009	-0.709				0.178	0	0.071																									
8862				0.758	0.937	-0.533				0.572	0	-0.178	2.131	2.453	0.324	0.920	0	-0.435																			
8891				-0.765	-1.616	-1.828																															
8898				0.838	0.182	0.470				1.133	0	0.071	-0.591	-0.703	-0.619	0.178	0	-0.435																			
9002				-0.656	0.514	-0.021				-1.308	0	1.424																									
9034																																					
9306													-1.223	0.100	-0.619	-0.194	0	-0.842																			
9408				2.333	-0.543	2.127				1.927	0	0.515	-0.535	0.795	0.949	-0.477	0	2.214																			
9436				-1.801	-0.543	0.787				-0.509	0	-0.454	-0.557	1.899	-0.619	-0.882	0	-0.076																			
9441													-0.602	-0.981	-0.906	-1.705	0	0.249																			
9524				0.803	0.982	-0.021				0.075	0	-0.454	0.942	0.323	-0.204	1.162	0	-0.435																			
9736													-0.381	0.165	-1.354	-0.664	0	-0.435																			
9899				0.696	0.465	0.630				0.262	0	-0.768	0.885	0.926	1.189	0.808	0	0.249																			
9903				0.917	1.146	1.249				0.279	0	0.071																									

 |z| ≥ 3,0 ("Unacceptable" or "Action")
 2,0 < |z| < 3,0 ("Warning")
 The parameter is not evaluated
 The result is not evaluated

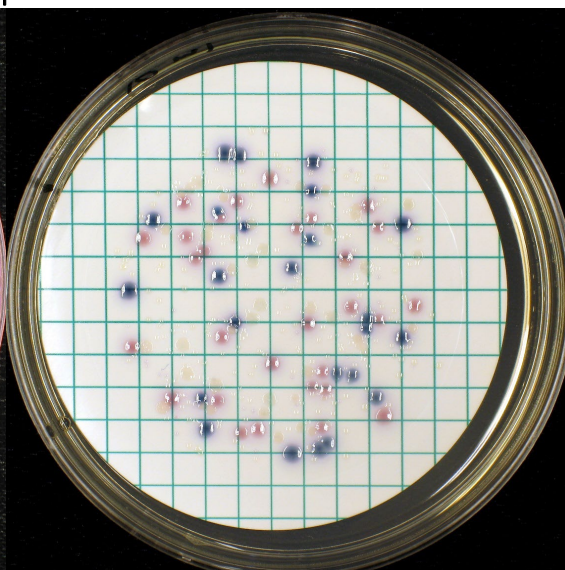
Sample A

m-Endo Agar LES, 37 °C



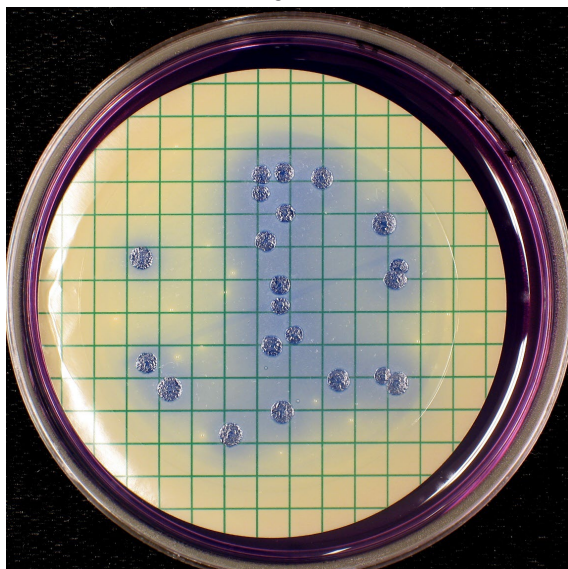
10 ml

Chromocult Coliform Agar, 37 °C



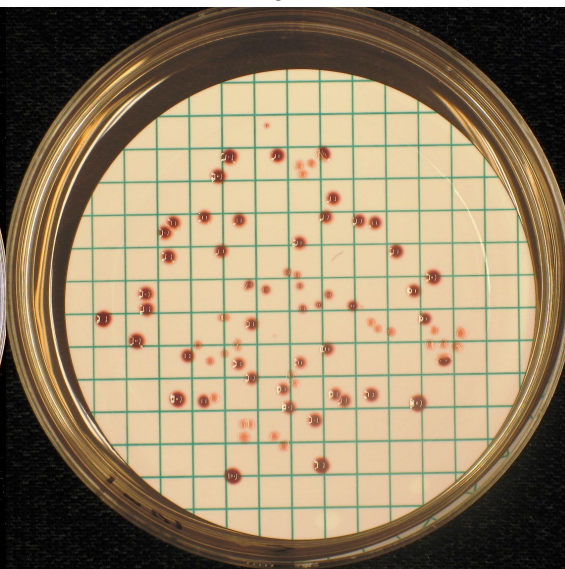
10 ml

m-FC Agar, 44 °C



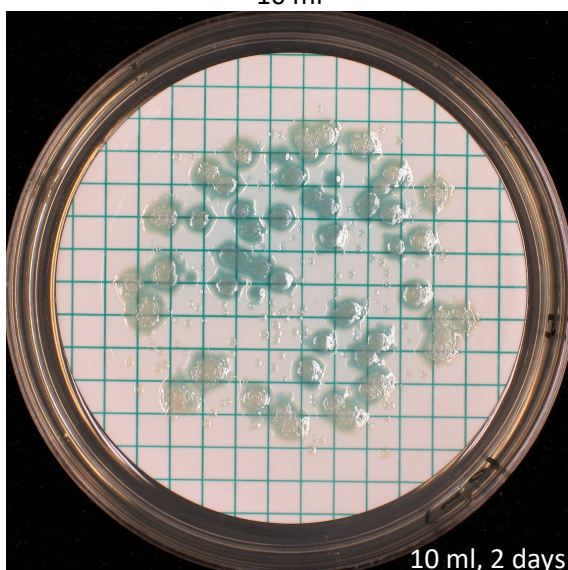
10 ml

m-Enterococcus Agar, 37 °C



10 ml

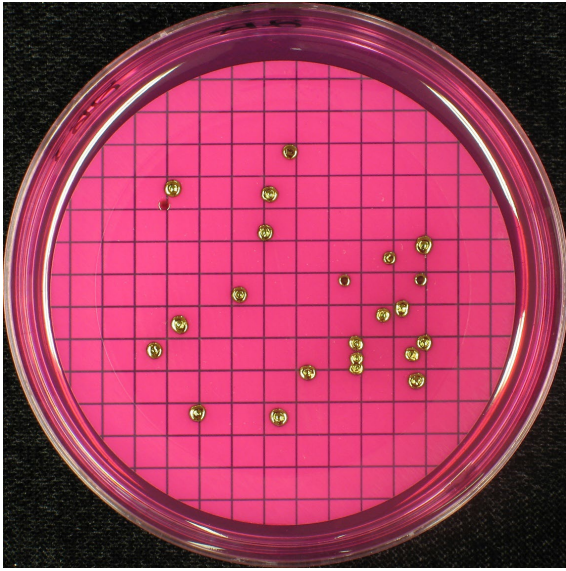
m-Pseudomonas CN Agar, 37 °C



10 ml, 2 days

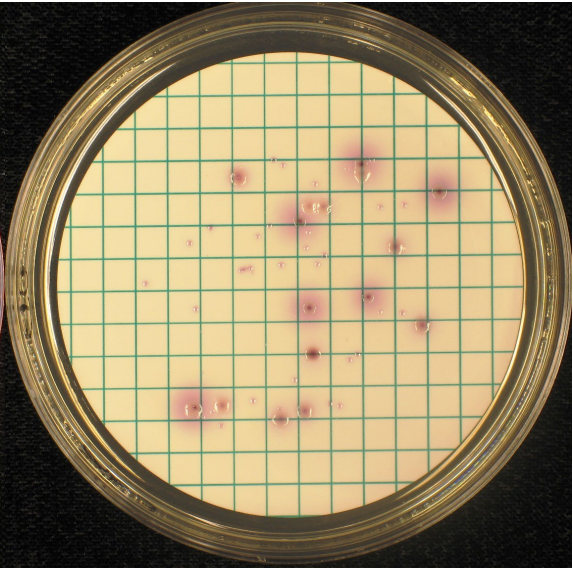
Sample B

m-Endo Agar LES, 37 °C



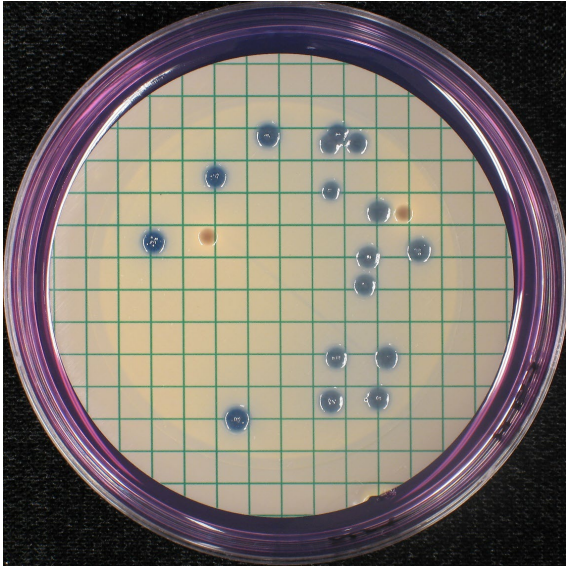
10 ml

Chromocult Coliform Agar, 37 °C



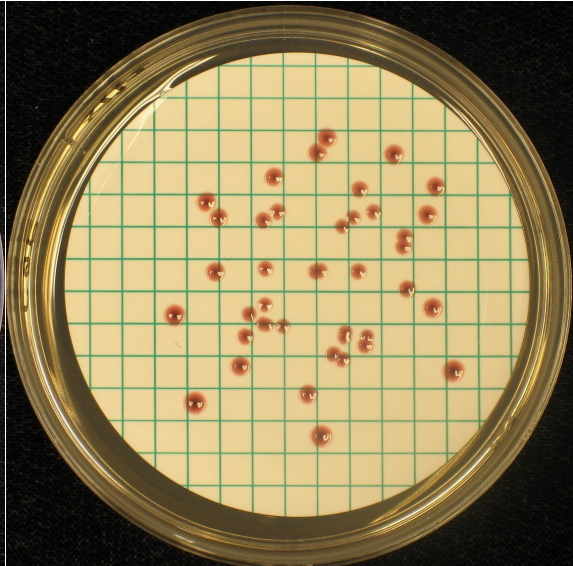
10 ml

m-FC Agar, 44 °C



100 ml

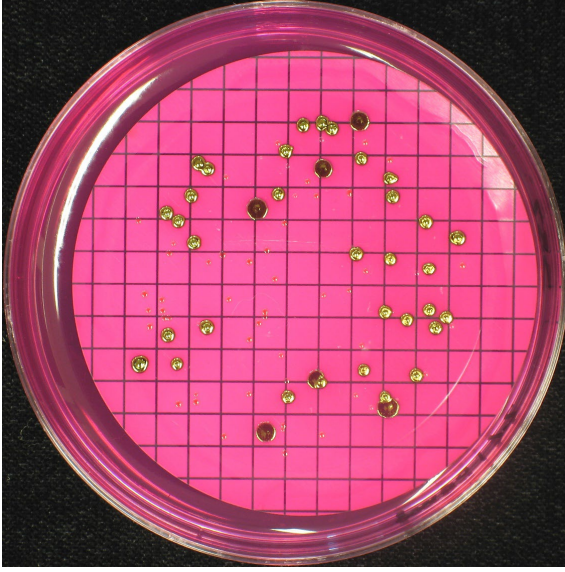
m-Enterococcus Agar, 37 °C



10 ml, 2 days

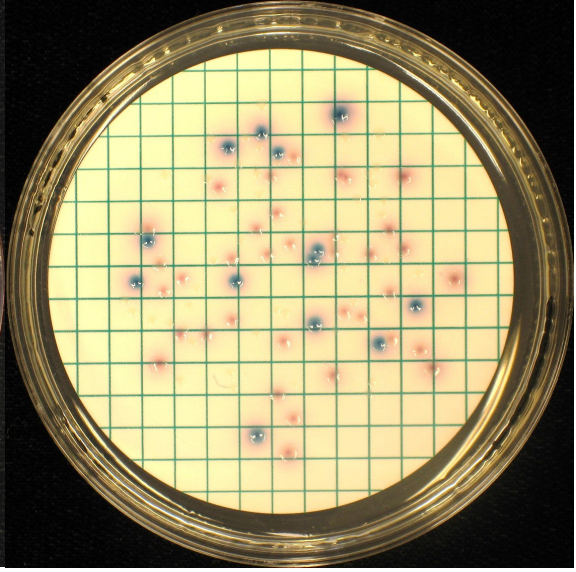
Sample C

m-Endo Agar LES, 37 °C



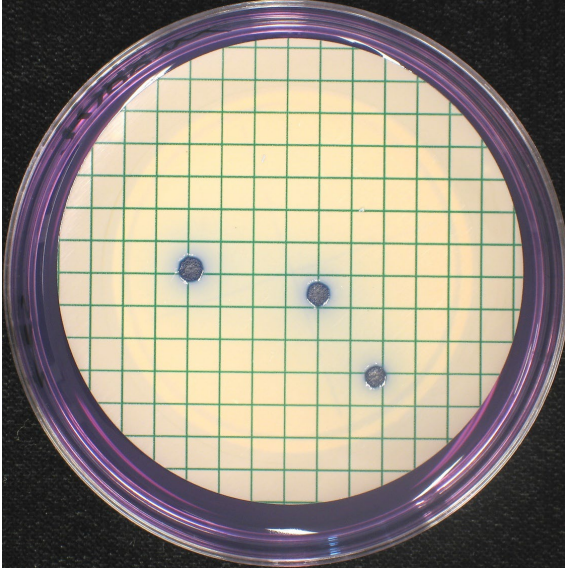
100 ml

Chromocult Coliform Agar, 37 °C



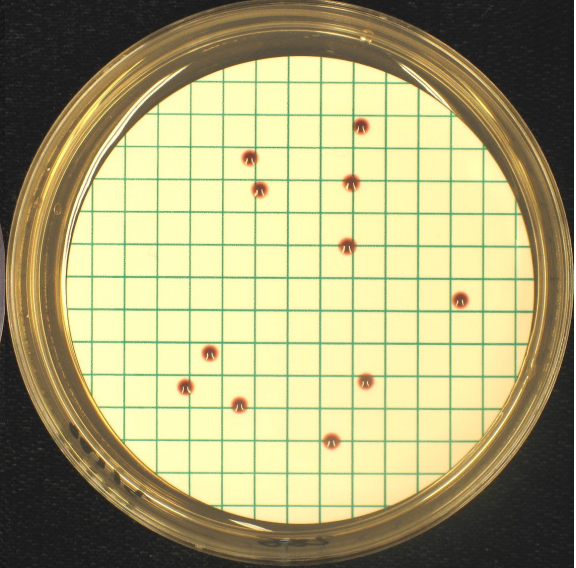
100 ml

m-FC Agar, 44 °C



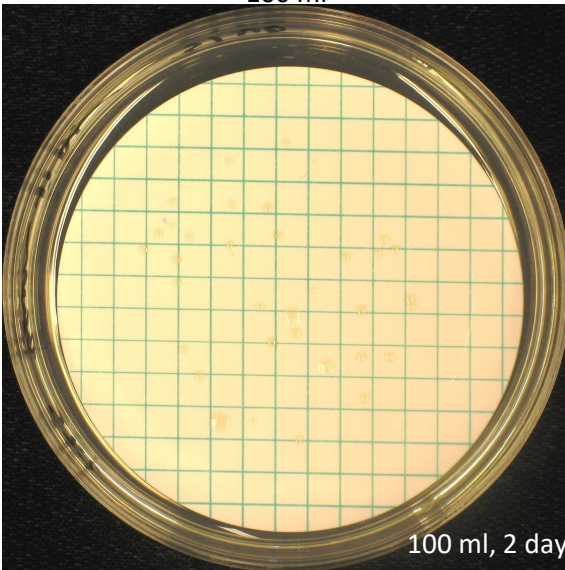
100 ml

m-Enterococcus Agar, 37 °C



100 ml

m-Pseudomonas CN Agar, 37



100 ml, 2 day

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