

# Proficiency testing Drinking water Microbiology

September 2024

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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,  $22 \pm 2$  °C for  $68 \pm 4$  hours

Culturable microorganisms,  $36 \pm 2$  °C for  $44 \pm 4$  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 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” or “< value” are not evaluated.

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 <sup>-1</sup> or cfu ml <sup>-1</sup> , re-transformed to the cfu / MPN scale
$Med$	median in cfu /MPN100 ml <sup>-1</sup>
$CV$	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 82 participants; 36 in Sweden, 45 in other European countries, and one outside of Europe. In total, 79 participants (96 %) reported results, of which 30 (38 %) provided at least one result with a remark.

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			
<b>Microorganisms</b>	<i>Escherichia coli</i> <i>Enterobacter cloacae</i> <i>Enterococcus faecalis</i> <i>Burkholderia cepacia</i>				<i>Escherichia coli</i> <i>Hafnia alvei</i> <i>Enterococcus faecium</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus capitis</i>				<i>Cronobacter sakazakii</i> <i>Aeromonas caviae</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus saprophyticus</i>			
<b>Analysis</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>
Coliform bacteria	<i>E. coli</i> <i>E. cloacae</i>	106	0	7	<i>E. coli</i> <i>H. alvei</i>	107	5	1	<i>C. sakazakii</i> ( <i>A. caviae</i> )	106	5	3
Suspected thermotolerant coliform bacteria	<i>E. coli</i> <i>E. cloacae</i>	18	0	0	<i>E. coli</i>	18	0	0	<i>C. sakazakii</i>	18	0	0
E. coli	<i>E. coli</i>	107	2	5	<i>E. coli</i>	108	4	4	-	107	1	0
Intestinal enterococci	<i>E. faecalis</i>	82	0	0	<i>E. faecium</i>	83	1	0	( <i>S. saprophyticus</i> )	83	1	0
P. aeruginosa	( <i>B. cepacia</i> )	53	3	0	<i>P. aeruginosa</i>	54	1	2	<i>P. aeruginosa</i>	54	0	3
Culturable microorganisms, 22 °C for 3 days	<i>All</i>	75	0	4	<i>E. faecium</i>	75	0	2	<i>S. saprophyticus</i>	75	0	1
Culturable microorganisms, 37 °C for 2 days	<i>All</i>	57	0	3	<i>S. capitis</i>	57	0	7	<i>S. saprophyticus</i>	57	0	1

- no target organism or no value

**microorganism** = main target organism

microorganism = The microorganism may give different results depending on method or definition used

(*microorganism*) = false positive before confirmation

The results are not evaluated

# Coliform bacteria

## Sample A

The strains of *E. coli* and *E. cloacae* 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 *E. cloacae* form blue and pink colonies, respectively. Both strains possess the enzyme  $\beta$ -galactosidase and are detected as coliform bacteria with Colilert/Colilert-18.

In total, 106 results were evaluated. Seven low outliers were reported from six participants. One low outlier may be due to the result being logarithmised.

## Sample B

The strains of *E. coli* and *H. alvei* were target organisms. *E. coli* possesses the enzyme  $\beta$ -galactosidase and forms typical colonies on most MF media at 35/36/37 °C. In comparison, *H. alvei* has a low  $\beta$ -galactosidase activity. It therefore forms beige to pale pink colonies on CCA and may require an extended incubation time for Colilert-18 (22 hours maximum incubation). On LES, *H. alvei* forms atypical red colonies without metallic sheen. Despite this, the mean was slightly higher with LES, and the strain appears to have been included by the participants.

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

In total, 107 results were evaluated. Five false negative results and one low outlier were identified.

The five false negative results were reported by four participants. They reported using CCA (unknown method) and an MPN-method with MacConkey broth.

## Sample C

The strain of *C. sakazakii* was target organism. *C. sakazakii* possesses the enzyme  $\beta$ -galactosidase and forms typical colonies on most MF media at 35/36/37 °C. The oxidase-positive strain of *A. caviae* was present as a false-positive organism for the analysis.

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

In total, 106 results were evaluated. Five false negative results, as well as one low and two high outliers were identified.

The false negative results were reported by four participants that used CCA according to either ISO 9308-1 or unknown methods, as well as one participant that used an MPN-method with MacConkey broth.

## General remarks

For MF methods, most participants 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,  $\beta$ -D-galactosidase positive (pink to red) colonies are counted as presumptive coliform bacteria.  $\beta$ -D-galactosidase and  $\beta$ -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 and confirmed 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 is done by reference to MPN tables. For MPN methods, most participants followed (EN) ISO 9308-2:2012 using Colilert-18. This was last reviewed by ISO in 2023 and remains current. As with CCA, Colilert-18 is based on the activity of  $\beta$ -D-galactosidase.  $\beta$ -D-galactosidase cleave ortho-nitrophenol galactoside (ONPG) and changes the coloration of the wells to yellow.

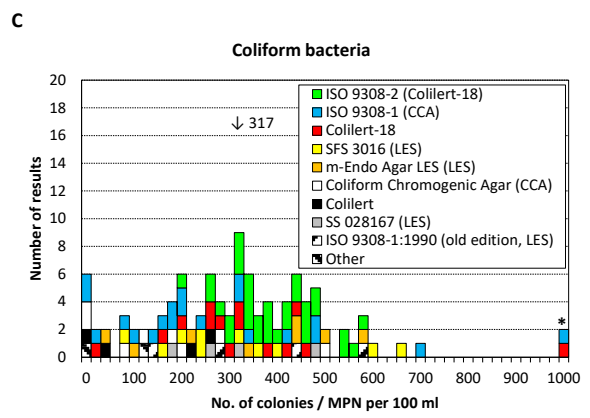
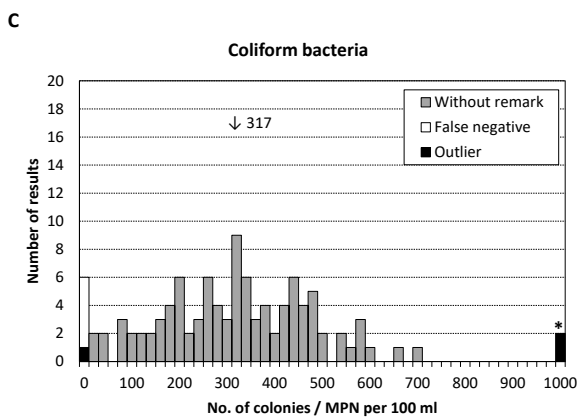
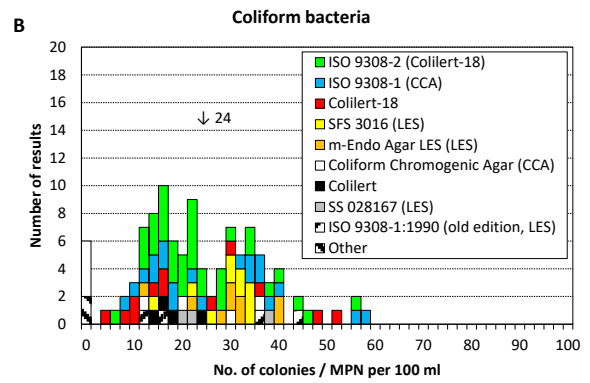
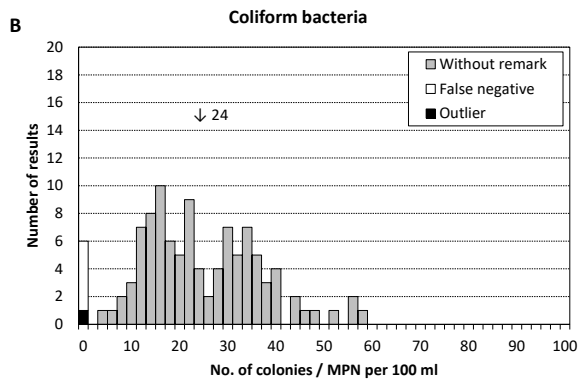
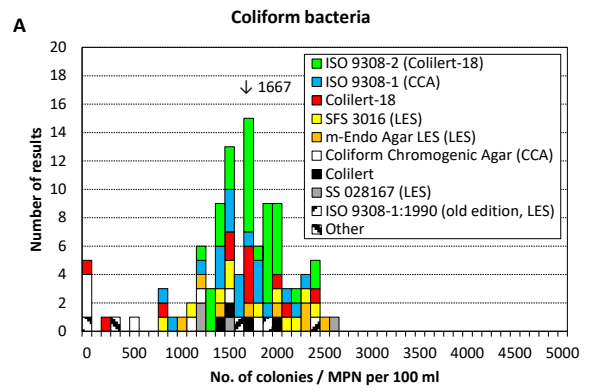
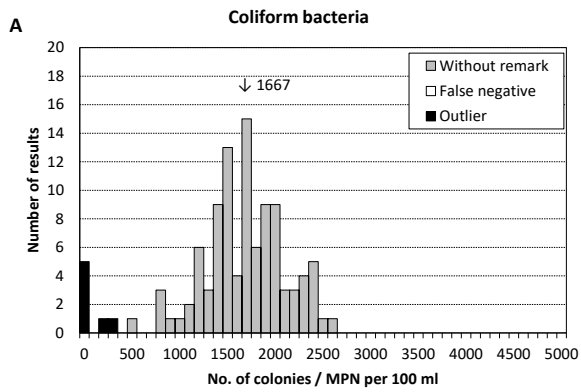
**Note:** Two participants reported the same set of result twice, together with the same method. Only one of these sets was evaluated. The other, marked in blue in Appendix 1 and 2, was excluded from the evaluation. One participant communicated that there was an error in their data submission. These results were therefore excluded from calculations of  $m_{PT}$  and  $s_{PT}$ , but were nevertheless included in the evaluation. This information is highlighted in blue text in Appendix 1.

**Table 2.** Results from analysis of coliform bacteria.

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	<	>
<b>All results</b>	<b>106</b>	<b>99</b>	<b>1667</b>	<b>14</b>	<b>0</b>	<b>7</b>	<b>0</b>	<b>107</b>	<b>101</b>	<b>24</b>	<b>25</b>	<b>5</b>	<b>1</b>	<b>0</b>	<b>106</b>	<b>98</b>	<b>317</b>	<b>26</b>	<b>5</b>	<b>1</b>	<b>2</b>
ISO 9308-2 (Colilert-18)	34	34	1730	9	0	0	0	35	35	22	23	0	0	0	32	32	382	12	0	0	0
ISO 9308-1 (CCA)	20	20	1600	12	0	0	0	20	20	23	27	0	0	0	20	17	209	35	2	0	1
Colilert-18	12	10	1736	13	0	2	0	12	12	16	35	0	0	0	14	13	310	25	0	0	1
SFS 3016 (LES)	11	11	1800	16	0	0	0	10	10	32	13	0	0	0	10	10	291	29	0	0	0
m-Endo Agar LES (LES)	8	8	1899	16	0	0	0	9	9	30	16	0	0	0	8	8	403	36	0	0	0
Coliform Chromogenic Agar	8	5	1270	20	0	3	0	8	4	-	-	3	1	0	8	6	195	32	2	0	0
Colilert	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	3	-	-	0	1	0
SS 028167 (LES)	4	4	-	-	0	0	0	3	3	-	-	0	0	0	4	4	-	-	0	0	0
ISO 9308-1:1990 (old edition, LES)	3	3	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Other	2	0	-	-	0	2	0	2	0	-	-	2	0	0	2	1	-	-	1	0	0

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

For individual methods,  $m_{PT}$  = median value in cfu / MPN 100 ml<sup>-1</sup>



**Figure 1.** Results from analysis of coliform bacteria

# 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. *E. cloacae* may also grow as suspected thermotolerant coliform bacterium at 44/44.5 °C with blue colonies.

In total, 18 results were reported.

## Sample B

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

In total, 18 results were reported.

## Sample C

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

In total, 18 results were reported.

## 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, 18 results were reported. Most participants used m-FC. The elevated incubation temperature (44/44.5 °C) and the addition of rosolic acid makes m-FC selective for thermotolerant coliform bacteria. ISO is currently developing a draft standard for membrane filtration method of *E. coli* in water with high levels of background bacteria.

**Table 3.** 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	<	>
<b>All results</b>	<b>18</b>	<b>18</b>	<b>652</b>	-	-	-	-	<b>18</b>	<b>18</b>	<b>9</b>	-	-	-	-	<b>18</b>	<b>18</b>	<b>34</b>	-	-	-	-
m-FC Agar (m-FC)	4	4	-	-	-	-	-	4	4	-	-	-	-	-	4	4	-	-	-	-	-
SFS 4088 (m-FC)	4	4	-	-	-	-	-	4	4	-	-	-	-	-	4	4	-	-	-	-	-
SS 028167 (m-FC)	3	3	-	-	-	-	-	3	3	-	-	-	-	-	3	3	-	-	-	-	-
Other	2	2	-	-	-	-	-	2	2	-	-	-	-	-	2	2	-	-	-	-	-
ISO 9308-1:1990 (old edition, m-FC)	2	2	-	-	-	-	-	2	2	-	-	-	-	-	2	2	-	-	-	-	-

MacConkey Agar	1	1	-	-	-	-	-	-	1	1	-	-	-	-	-	1	1	-	-	-	-	-
Coliform Chromogenic Agar	1	1	-	-	-	-	-	-	1	1	-	-	-	-	-	1	1	-	-	-	-	-
NS 4792 (m-FC)	1	1	-	-	-	-	-	-	1	1	-	-	-	-	-	1	1	-	-	-	-	-

Med= Median value in cfu 100 ml<sup>-1</sup>

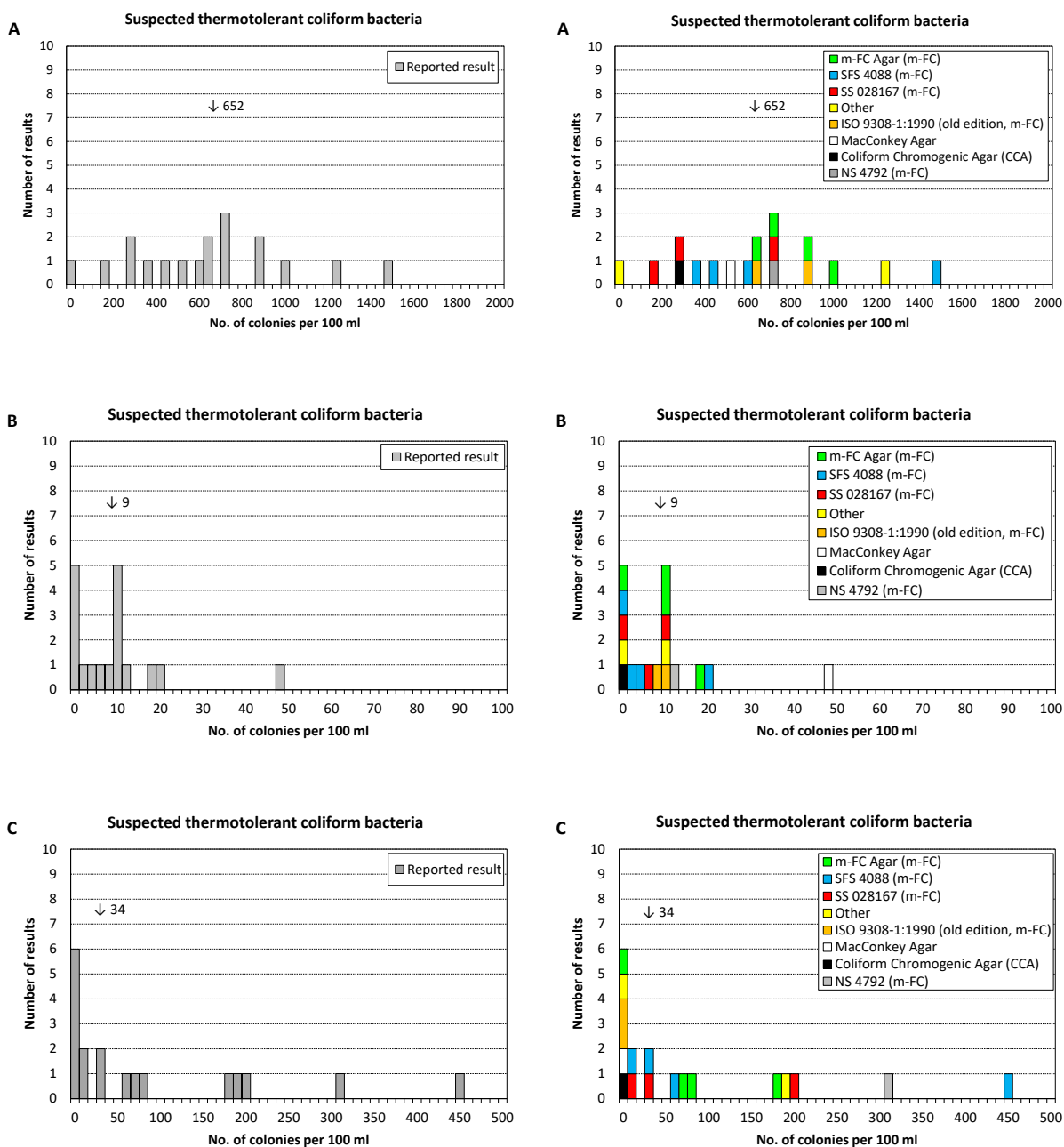


Figure 2. Results from analysis of suspected thermotolerant coliform bacteria

# *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  $\beta$ -glucuronidase and is detected as *E. coli* with Colilert/Colilert-18. The strain is positive for indole production and produces gas in Lactose-Tryptone-Lauryl Sulphate Broth (LTLSB).

In total, 107 results were evaluated. Two false negative results, as well as one high and four low outliers were identified.

The false negative results were reported by a single participant, using an MF-method and an MPN-method. One of the low outliers may be due to the result having been logarithmised.

## Sample B

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 produces gas in LTLSB.

In total, 108 results were evaluated. Four false negative results, as well as two high and two low outliers were identified.

The false negative results were reported by three participants that used CCA (unknown methods), Colilert according to the manufacturer's instructions, and an MPN-method with MacConkey broth.

## Sample C

No target organism was present in the sample.

In total, 107 results were evaluated. One false positive result was reported by a participant that used LES (unknown method).

## General remarks

Most participants followed (EN) ISO 9308-2:2012, (EN) ISO 9308-1:2014 and/or Nordic national standards (see table 4). (EN) ISO 9308 defines *E. coli* as a member of the Enterobacteriaceae that possesses both  $\beta$ -D-galactosidase and  $\beta$ -D-glucuronidase enzymes. On CCA,  $\beta$ -D-galactosidase and  $\beta$ -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  $\beta$ -D-glucuronidase, some participants have modified their standard accordingly. Depending on the method, tests for gas production, indole production and/or  $\beta$ -glucuronidase activity are usually performed from oxidase-negative presumptive colonies.

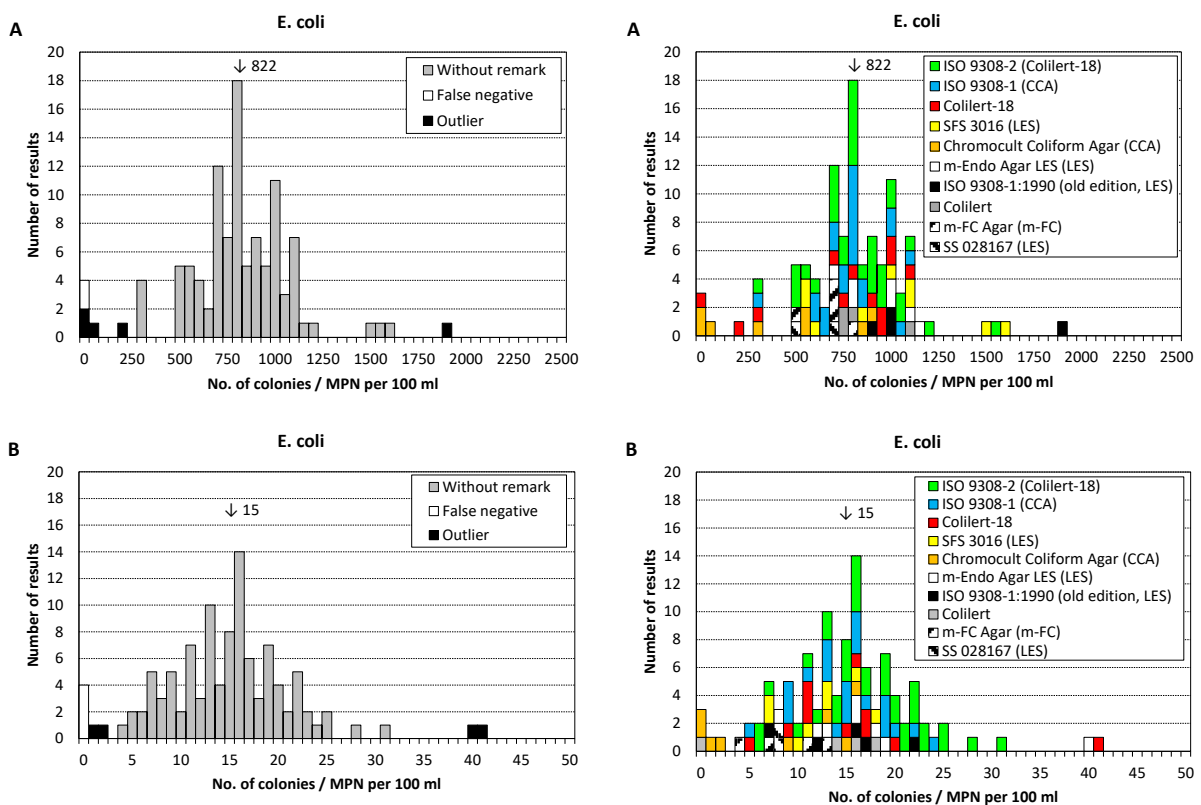
The primary MF growth media CCA and LES are incubated at 35/36/37 °C and m-FC at 44/44.5 °C.

**Note:** Two participants reported the same set of result twice, together with the same method. Only one of these sets was evaluated. The other, marked in blue in Appendix 1 and 2, was excluded from the evaluation. One participant communicated that there was an error in their data submission. These results were therefore excluded from calculations of  $m_{PT}$  and  $s_{PT}$ , but were nevertheless included in the evaluation. This information is highlighted in blue text in Appendix 1.

**Table 4.** Results from analysis of *Escherichia coli*.

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	<	>
<b>All results</b>	<b>107</b>	<b>100</b>	<b>822</b>	<b>13</b>	<b>2</b>	<b>4</b>	<b>1</b>	<b>108</b>	<b>100</b>	<b>15</b>	<b>20</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>107</b>	<b>106</b>	-	-	<b>1</b>	-	-
ISO 9308-2 (Colilert-18)	33	33	818	14	0	0	0	35	35	17	18	0	0	0	32	32	-	-	0	-	-
ISO 9308-1 (CCA)	22	22	805	11	0	0	0	22	22	15	17	0	0	0	22	22	-	-	0	-	-
Colilert-18	12	10	939	15	0	2	0	11	10	13	18	0	0	1	14	14	-	-	0	-	-
SFS 3016 (LES)	9	9	1000	20	0	0	0	8	8	12	17	0	0	0	8	8	-	-	0	-	-
Chromocult Coliform Agar	8	5	570	22	1	2	0	8	4	-	-	2	2	0	8	8	-	-	0	-	-
m-Endo Agar LES (LES)	7	7	1000	9	0	0	0	8	7	16	14	0	0	1	8	7	-	-	1	-	-
ISO 9308-1:1990 (old edition, LES)	4	3	-	-	0	0	1	5	5	16	20	0	0	0	5	5	-	-	0	-	-
Colilert	4	4	-	-	0	0	0	4	3	-	-	1	0	0	4	4	-	-	0	-	-
m-FC Agar (m-FC)	5	5	720	8	0	0	0	4	4	-	-	0	0	0	3	3	-	-	0	-	-
SS 028167 (LES)	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	-	-
Other	1	0	-	-	1	0	0	1	0	-	-	1	0	0	1	1	-	-	0	-	-

For “All results”,  $m_{PT}$  = assigned value, robust mean value in cfu 100 ml<sup>-1</sup>, re-transformed to the cfu scale. For individual methods,  $m_{PT}$  = median value in cfu 100 ml<sup>-1</sup>. \*m-FC Agar (m-FC) includes the reporting of SS 028167 (m-FC), SFS 4088 (m-FC), NS 4792 (m-FC) and m-FC Agar (unknown method).



**Figure 3.** Results from analysis of *Escherichia coli*. The group “Other” is not shown in the figure.



# Intestinal enterococci

## Sample A

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

In total, 82 results were evaluated. No outliers were identified.

## Sample B

The strain of *E. faecium* was target organism. On m-Ent, it forms light red to maroon colonies. Upon confirmation on BEAA, the blackening may be partially weak (see Appendix 3). Due to the potential difficulties with the confirmation, no results are considered as outliers, and all positive results are considered acceptable. As a consequence, the z-scores in Appendix 2 should be interpreted with caution.

In total, 83 results were reported. One false negative result was identified.

## Sample C

No target organism was present in the sample. The strain of *S. saprophyticus* was present as a false-positive organism for the analysis. On m-Ent, it forms small light red to maroon colonies after two days. Upon confirmation on BEAA, no blackening is seen.

In total, 83 results were evaluated. One false positive result was identified.

## General remarks

Most participants followed (EN) ISO 7899-2:2000 using m-Ent (Slanetz & Bartley). With this 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. ISO 7899-2:2000 was last reviewed and confirmed by ISO in 2021 and remains current.

For MPN methods, 15 results were reported using Enterolert-E and ten results were reported using Enterolert-DW. The Draft International Standard (DIS), ISO/ DIS 7899-3, using Enterolert-DW, is currently in the enquiry phase with ISO members. The Enterolert-DW test defines intestinal enterococci as bacteria that are capable of growth in the defined substrate medium, and that produce a green color through cleavage of ortho-nitrophenyl- $\beta$ -D-glucoside by the enzyme  $\beta$ -D-glucosidase.

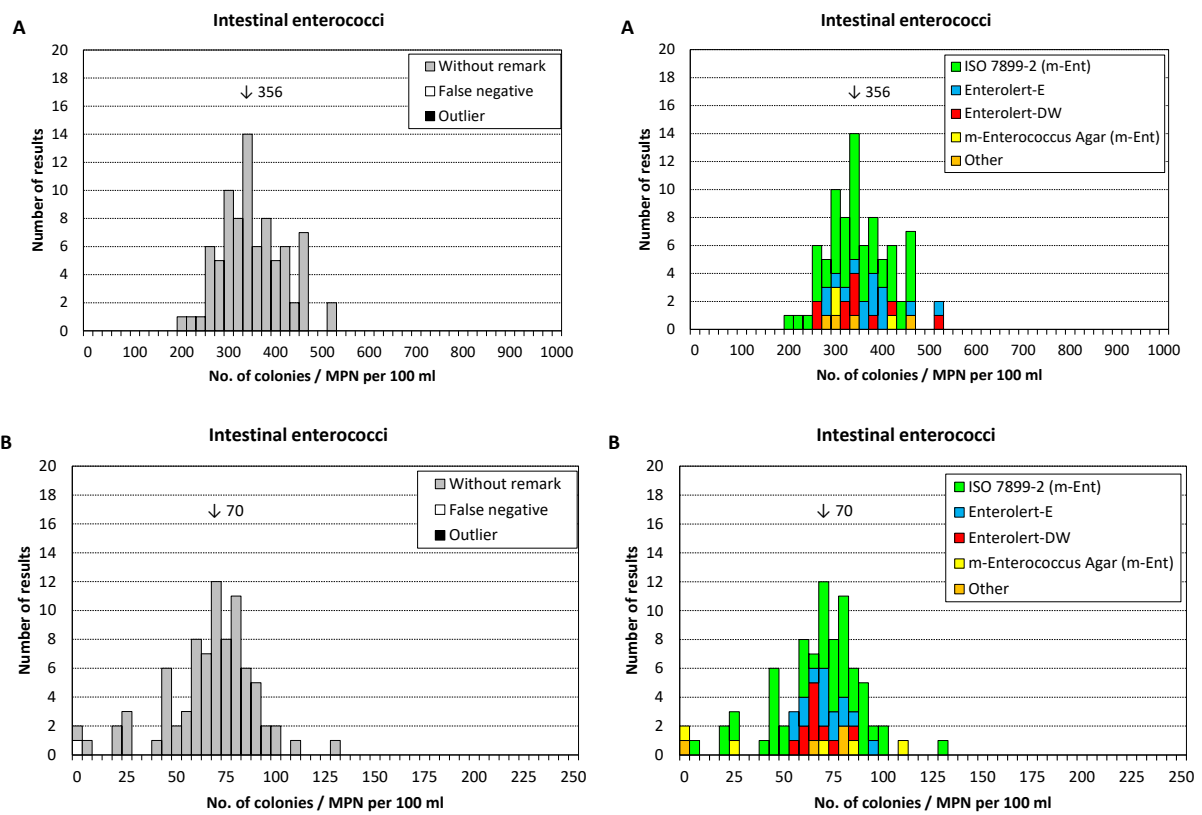
The primary MF growth media m-Ent is incubated at 35/36/37 °C and Enterolert-E and -DW at 41 °C.

**Table 5.** Results from analysis of intestinal enterococci.

Method	Sample A							Sample B							Sample C						
	N	n	m <sub>PT</sub>	CV	F	<	>	N	n	m <sub>PT</sub>	CV	F	<	>	N	n	m <sub>PT</sub>	CV	F	<	>
<b>All results</b>	<b>82</b>	<b>82</b>	<b>356</b>	<b>10</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>83</b>	<b>82</b>	<b>70</b>	<b>14</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>83</b>	<b>82</b>	-	-	<b>1</b>	-	-
ISO 7899-2 (m-Ent)	50	50	350	10	0	0	0	49	49	74	20	0	0	0	46	46	-	-	0	-	-
Enterolert-E	15	15	382	9	0	0	0	15	15	71	8	0	0	0	15	15	-	-	0	-	-
Enterolert-DW	10	10	344	10	0	0	0	10	10	67	6	0	0	0	10	10	-	-	0	-	-
m-Enterococcus Agar (m-Ent)	3	3	-	-	0	0	0	5	5	73	48	0	0	0	8	7	-	-	1	-	-
Other	4	4	-	-	0	0	0	4	3	-	-	1	0	0	4	4	-	-	0	-	-

For “All results”,  $m_{PT}$  = assigned value, robust mean value in cfu / MPN 100 ml<sup>-1</sup>, re-transformed to the cfu / MPN scale

For individual methods,  $m_{PT}$  = median value in cfu /MPN 100 ml<sup>-1</sup>



**Figure 4.** Results from analysis of intestinal enterococci.

# *Pseudomonas aeruginosa*

## Sample A

No target organism was present in the sample. On Pseudomonas Agar base/CN agar (PACN), *B. cepacia* may form transparent colonies.

In total, 53 results were evaluated. Three false positive results were identified.

## Sample B

The strain of *P. aeruginosa* was target organism. On PACN, it forms yellow-green colonies that fluoresce under UV light.

In total, 54 results were evaluated. One false negative result was identified, as well as one high and one low outlier.

## Sample C

The strain of *P. aeruginosa* was target organism. On PACN, it forms typical blue-green colonies that fluoresce under UV light.

In total, 54 results were evaluated. One low and two high outliers were identified.

## General remarks

Most participants followed (EN) ISO 16266:2006. With this standard, *P. aeruginosa* is defined as microorganisms that grow on selective media containing ceftrimide and produce pyocyanin, or microorganisms that grow on selective media containing ceftrimide, are oxidase positive, fluoresce under UV radiation, and are able to produce ammonia from acetamide. Since unhealthy substances are included in the confirmation test, some 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. ISO 16266:2006 was last reviewed and confirmed by ISO in 2021 and remains current.

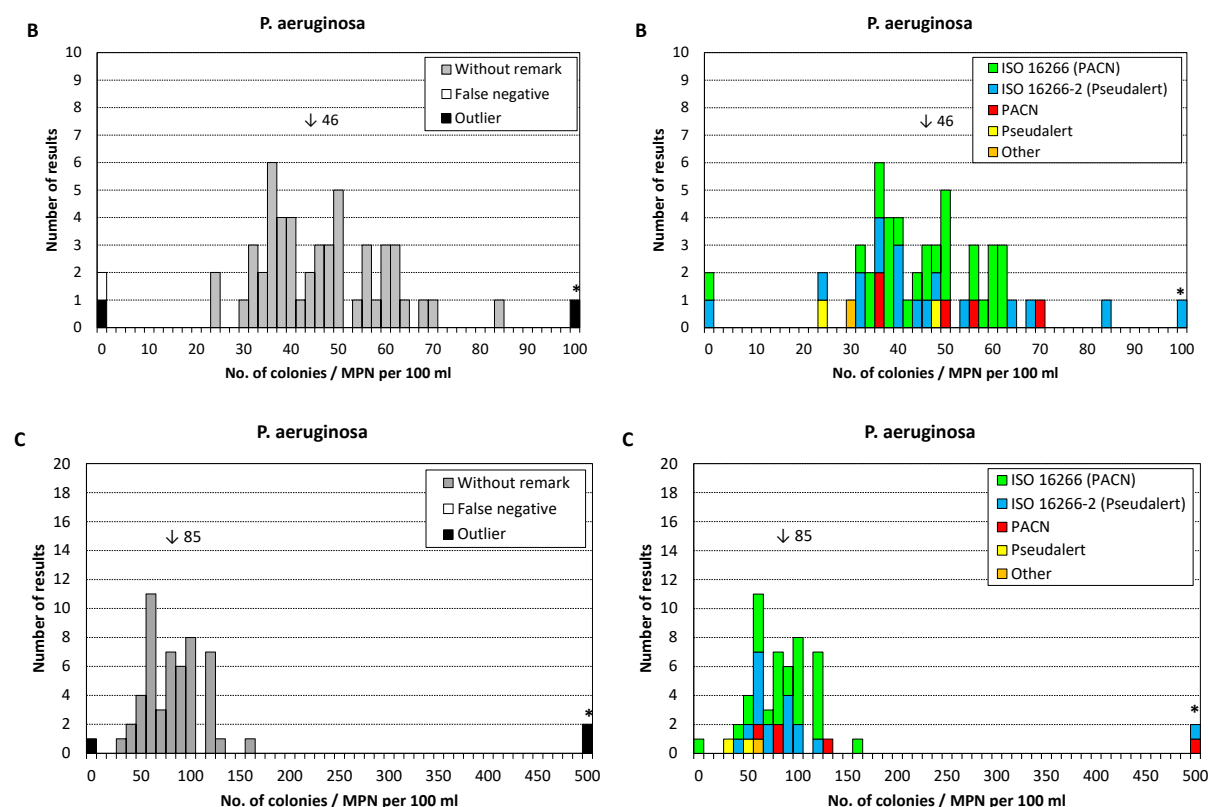
Seventeen results were reported by participants that followed EN ISO 16266-2:2018, which uses Pseudalert. The method is based on the growth of target organisms in a liquid medium and calculation of the MPN of organisms by reference to MPN tables. The *P. aeruginosa* enzyme aminopeptidase hydrolyses the substrate 7-amino-4-methylcoumarin and the positive wells for *P. aeruginosa* exhibit blue fluorescence under UV light. ISO 16266-2:2018 was last reviewed and confirmed by ISO in 2024 and remains current.

**Table 6.** Results from analysis of *P. aeruginosa*.

Method	Sample A						Sample B						Sample C					
	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>CV</i>	<i>F</i>	< >	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>CV</i>	<i>F</i>	< >	<i>N</i>	<i>n</i>	<i>m</i> <sub>PT</sub>	<i>CV</i>	<i>F</i>	< >
All results	53	50	-	-	3	- -	54	51	46	14	1	1 1	54	51	85	18	0	1 2
ISO 16266 (PACN)	29	28	-	-	1	- -	29	28	47	11	1	0 0	29	28	91	15	0	1 0
ISO 16266-2 (Pseudalert)	16	15	-	-	1	- -	17	15	41	16	0	1 1	17	16	74	14	0	0 1
PACN	5	4	-	-	1	- -	5	5	51	14	0	0 0	5	4	-	-	0	0 1
Pseudalert	2	2	-	-	0	- -	2	2	-	-	0	0 0	2	2	-	-	0	0 0
Other	1	1	-	-	0	- -	1	1	-	-	0	0 0	1	1	-	-	0	0 0

For "All results", *m*<sub>PT</sub> = assigned value, robust mean value in cfu / MPN 100 ml<sup>-1</sup>, re-transformed to the cfu / MPN scale

For individual methods, *m*<sub>PT</sub> = median value in cfu / MPN 100 ml<sup>-1</sup>



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

# Culturable microorganisms, $22 \pm 2$ °C for $68 \pm 4$ hours

## Sample A

All the strains included in the sample grow as culturable microorganisms at  $22 \pm 2$  °C.

In total, 75 results were evaluated. Two low and two high outliers were identified.

## Sample B

The few colonies that might occur consist mainly of *E. faecium*. The strain of *S. capitis* does not grow at  $22 \pm 2$  °C.

The concentration of culturable microorganisms was low; the  $m_{PT}$  was 2 cfu ml<sup>-1</sup>. A reported result of <1 cfu ml<sup>-1</sup> was included in the expected results range and is considered acceptable. Z-scores down to -3 are also considered acceptable.

In total, 75 results were evaluated. Two high outliers were identified.

## Sample C

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

In total, 75 results were evaluated. One low outlier was identified.

## General remarks

Most participants followed (EN) ISO 6222:1999, which describes a pour-plate method with Yeast extract Agar (YeA). With this standard, culturable microorganisms are defined as all aerobic bacteria, yeasts and moulds that are capable of forming colonies in the medium. ISO 6222:1999 was last reviewed and confirmed by ISO in 2021 and remains current. Some laboratories have modified the standard, and use Plate Count Agar (PCA) instead of YeA. No apparent differences between these media can be observed here. The high CV for PCA in sample B is not statistically relevant, as it is mainly a consequence of the very low concentration of target organisms.

**Note:** Two participants reported the same set of result twice, together with the same method. Only one of these sets was evaluated. The other, marked in blue in Appendix 1 and 2, was excluded from the evaluation.

**Table 7.** Results from analysis of culturable microorganisms,  $22 \pm 2$  °C for  $68 \pm 4$  hours.

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	<	>	
<b>All results</b>	<b>75</b>	<b>71</b>	<b>26</b>	<b>11</b>	<b>0</b>	<b>2</b>	<b>2</b>	<b>75</b>	<b>73</b>	<b>2</b>	<b>43</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>75</b>	<b>74</b>	<b>37</b>	<b>8</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>
ISO 6222 (YeA, pour plate)	46	46	26	9	0	0	0	46	44	2	36	0	0	2	46	46	39	7	0	0	0	0
Yeast extract Agar (YeA, pour plate)	9	8	25	17	0	0	1	10	10	2	53	0	0	0	10	10	34	8	0	0	0	0

ISO 6222 mod. (PCA, pour plate)	8	8	25	6	0	0	0	7	7	1	96	0	0	0	8	8	38	12	0	0	0
Plate Count Agar (PCA, pour plate)	5	3	-	-	0	1	1	5	5	0	224	0	0	0	4	3	-	-	0	1	0
Other	3	3	-	-	0	0	0	3	3	-	-	0	0	0	3	3	-	-	0	0	0
3M™ Petrifilm™ AC Plate	2	2	-	-	0	0	0	2	2	-	-	0	0	0	2	2	-	-	0	0	0
EasyDisc YEA Test	1	0	-	-	0	1	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0
Reasoner's 2A agar	1	1	-	-	0	0	0	1	1	-	-	0	0	0	1	1	-	-	0	0	0

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

For individual methods,  $m_{PT}$  = median value in cfu ml<sup>-1</sup>

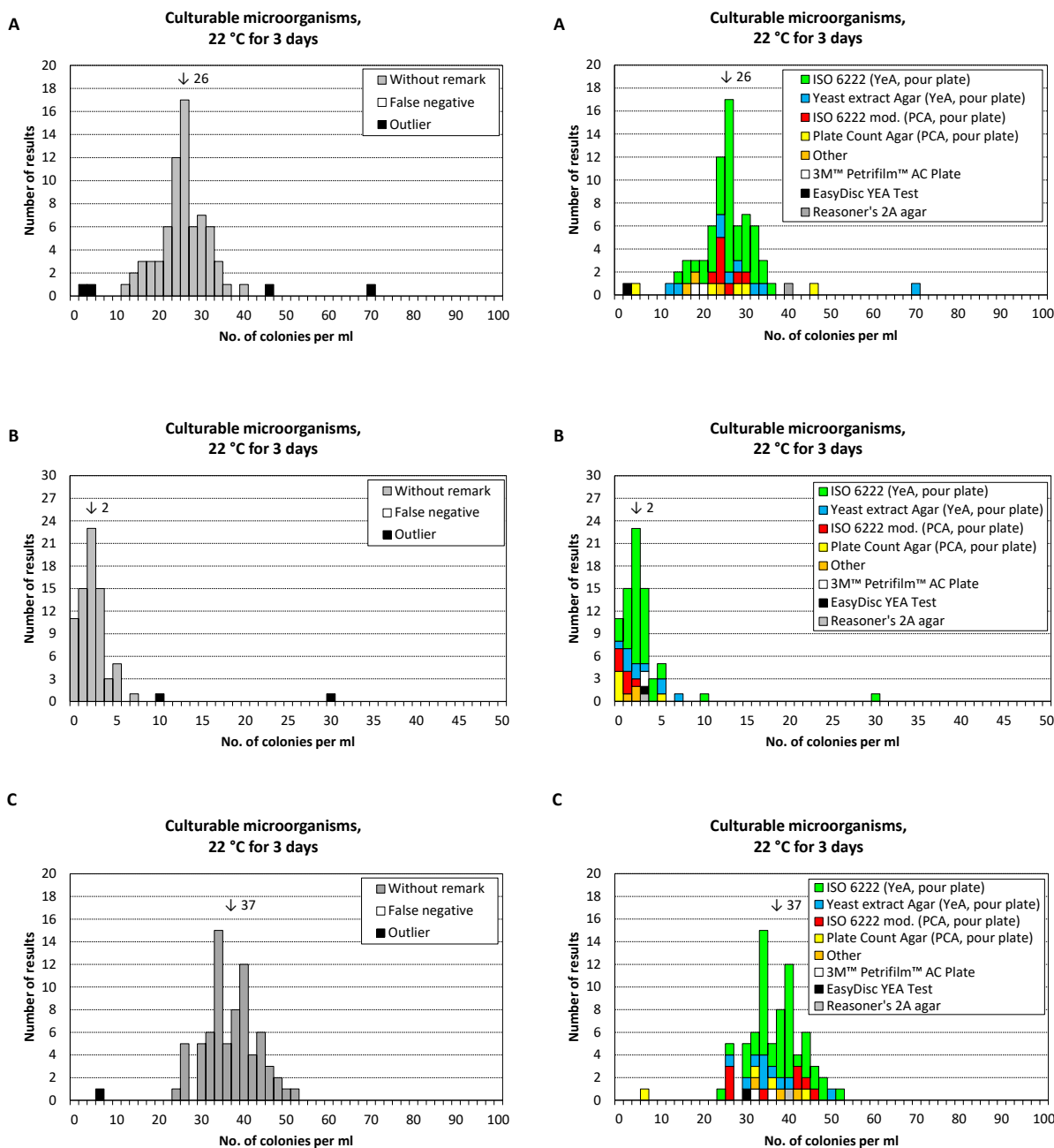


Figure 6. Results from analysis of culturable microorganisms, 22 ± 2 °C for 68 ± 4 hours.

# Culturable microorganisms, $36 \pm 2$ °C for $44 \pm 4$ hours

## Sample A

All the strains in the sample grow as culturable microorganisms at  $36 \pm 2$  °C.

In total, 57 results were evaluated. One low and two high outliers were identified.

## Sample B

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

In total, 57 results were evaluated. One high and six low outliers were identified.

## Sample C

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

In total, 57 results were evaluated One high outlier was identified.

## General remarks

Most participants followed the standard (EN) ISO 6222:1999, occasionally modified to use PCA instead of YeA. Sample B included the strain *S. capitis*, which grows at  $36 \pm 2$  °C but not at  $22 \pm 2$  °C. As previously observed with this strain, there are some inexplicable low results.

**Note:** One participant reported the same set of result twice, together with the same method. Only one of these sets was evaluated. The other, marked in blue in Appendix 1 and 2, was excluded from the evaluation.

**Table 8.** Results from analysis of culturable microorganisms,  $36 \pm 2$  °C for  $44 \pm 4$  hours.

Method	Sample A								Sample B								Sample C							
	N	n	m <sub>PT</sub>	CV	F	<	>		N	n	m <sub>PT</sub>	CV	F	<	>		N	n	m <sub>PT</sub>	CV	F	<	>	
<b>All results</b>	57	54	26	12	0	1	2		57	50	60	12	0	6	1		57	56	38	8	0	0	1	
ISO 6222 (YeA, pour plate)	35	34	27	10	0	0	1		35	30	63	7	0	4	1		34	34	40	7	0	0	0	
ISO 6222 mod. (PCA, pour plate)	8	7	25	13	0	0	1		8	8	52	18	0	0	0		9	8	38	10	0	0	1	
Yeast extract Agar (YeA, pour plate)	7	7	23	18	0	0	0		7	6	61	12	0	1	0		7	7	40	10	0	0	0	
Plate Count Agar (PCA, pour plate)	3	2	-	-	0	1	0		3	2	-	-	0	1	0		3	3	-	-	0	0	0	
Other	2	2	-	-	0	0	0		2	2	-	-	0	0	0		2	2	-	-	0	0	0	
3M™ Petrifilm™ AC Plate	2	2	-	-	0	0	0		2	2	-	-	0	0	0		2	2	-	-	0	0	0	

For "All results", m<sub>PT</sub> = assigned value, robust mean value in cfu ml<sup>-1</sup>, re-transformed to the cfu scale

For individual methods, m<sub>PT</sub> = median value in cfu ml<sup>-1</sup>

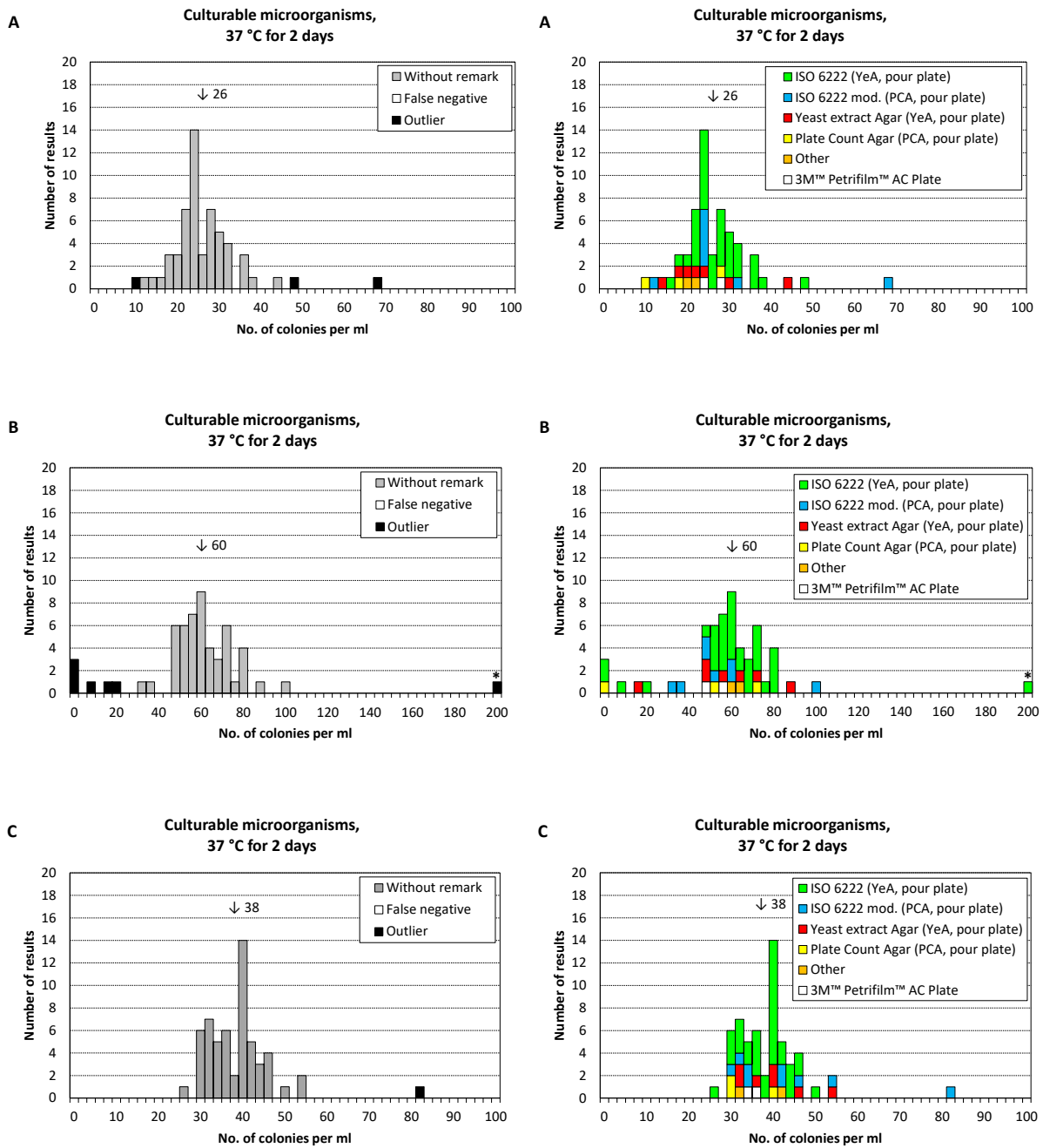


Figure 7. Results from analysis of culturable microorganisms,  $36 \pm 2$  °C for  $44 \pm 4$  hours.



# 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.

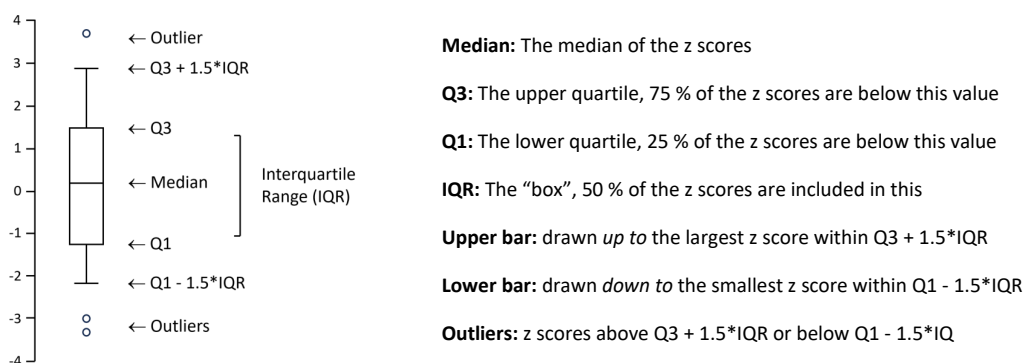
Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol [2].

Samples for follow-up analyses can be ordered at: <https://laboratory.livsmedelsverket.se>

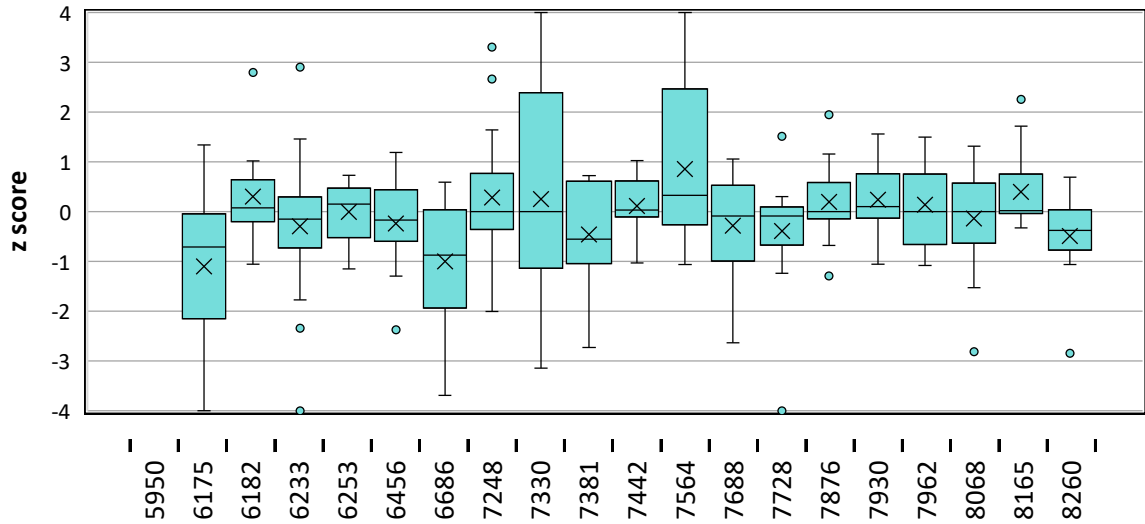
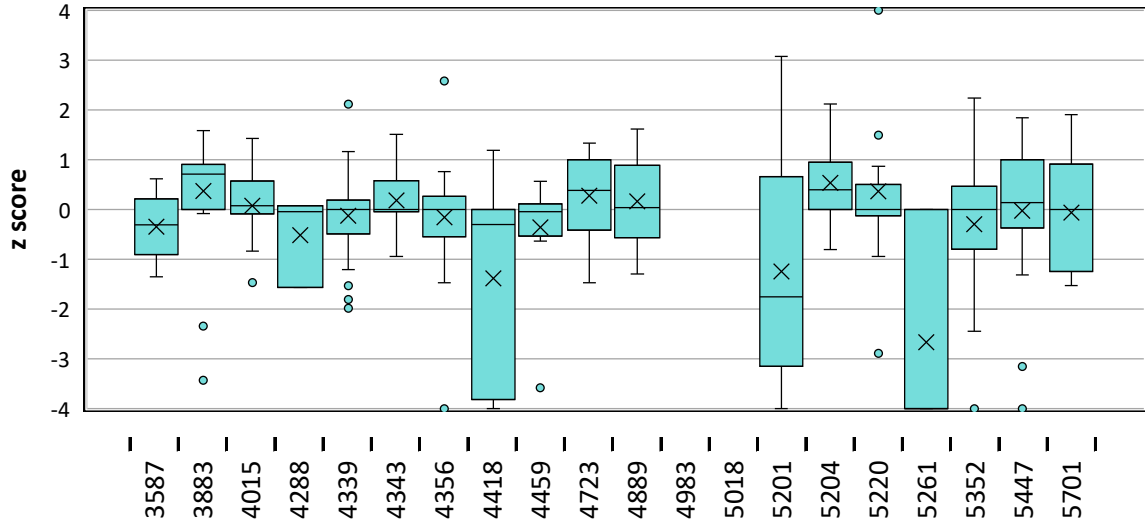
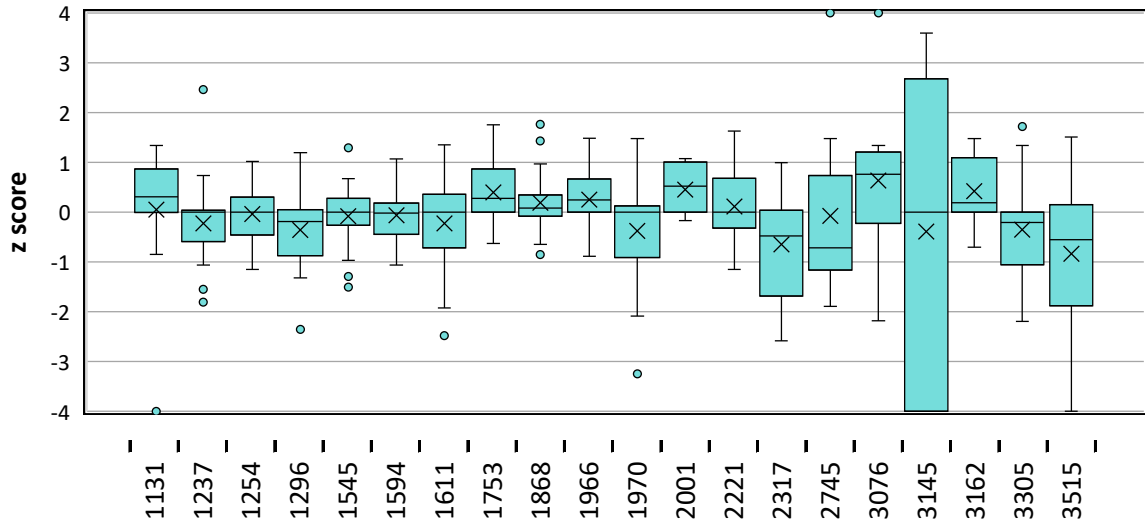
## Box plots and numbers of deviating results for each participant

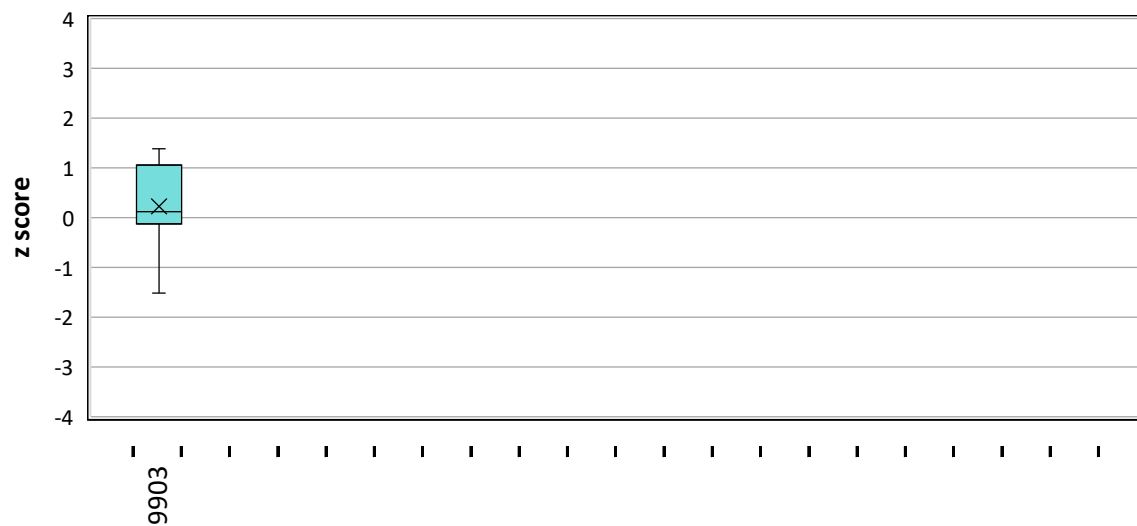
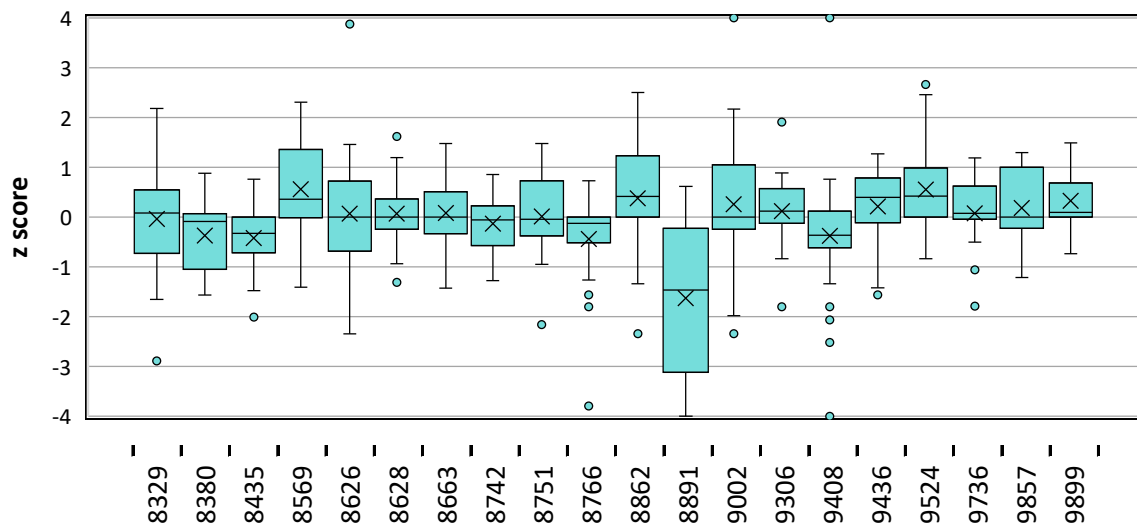
Box plots are based on the  $z$ -scores listed in Appendix 2 and give a comprehensive view of the performance of each participant. The range of  $z$ -scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred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

The different parts of a box plot are shown in figure 8.



**Figure 8.** Schematic explanation of a box plot.





# Test material and quality control

## Test material

Each participant received three samples with freeze-dried microorganisms, designated A–C. The test material was freeze-dried in 0.5 ml portions in glass vials, as described by Peterz and Steneryd [3]. Before analysing the samples, the contents of each vial should be reconstituted in 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 9.** Microorganisms and approximate concentrations in the samples.

Sample	Microorganism	Strain			cfu/100 ml <sup>3</sup>
		SLV no. <sup>1</sup>	Origin	Reference <sup>2</sup>	
A	<i>Escherichia coli</i>	SLV-165	Drinking water	CCUG 43 600	1017
	<i>Enterobacter cloacae</i>	SLV-451	-	CCUG 30 205	1165
	<i>Enterococcus faecalis</i>	SLV-051	-	CCUG 45 101	407
	<i>Burkholderia cepacia</i>	SLV-042	-	-	597
B	<i>Escherichia coli</i>	SLV-082	Drinking water	CCUG 45 097	15
	<i>Hafnia alvei</i>	SLV-566	Water	-	26
	<i>Enterococcus faecium</i>	SLV-459	-	CCUG 35 172	86
	<i>Pseudomonas aeruginosa</i>	SLV-455	-	CCUG 30 209	60
	<i>Staphylococcus capitis</i>	SLV-463	-	CCUG 35 173	61*
C	<i>Cronobacter sakazakii</i>	SLV-419	Untreated water	-	317
	<i>Aeromonas caviae</i>	SLV-533	Water	CCUG 48 892	148
	<i>Pseudomonas aeruginosa</i>	SLV-395	Drinking water	CCUG 43 596	114
	<i>Staphylococcus saprophyticus</i>	SLV-013	-	CCUG 45 100	35*

<sup>1</sup> Internal strain identification no. at the Swedish Food Agency

<sup>2</sup> Culture collection: CCUG: Culture Collection University of Gothenburg

<sup>3</sup> cfu = colony forming units

\* indicates cfu per ml

## Quality control of the samples

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

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

$s_R$ : the expected laboratory variation, generally assumed to be 1.25 for the Drinking water scheme.

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

**Table 10.** Concentration mean ( $m$ ), between-vial variation ( $s_{bb}$ ) and  $p$  values from the quality control of the samples;  $m$  is expressed in square root cfu (colony forming units) per 100 ml of sample for MF methods and per 1 ml for pour plate methods.

Analysis and method	A <sup>1</sup>			B <sup>1</sup>			C <sup>1</sup>		
	$m$	$s_{bb}$	$p$	$m$	$s_{bb}$	$p$	$m$	$s_{bb}$	$p$
Coliform bacteria (MF) SS-EN ISO 9308-1:2014	4.70 <sup>2</sup>	0.18	0.37	6.45	0.40	0.15	3.98 <sup>4</sup>	0.00	0.68
Suspected thermotolerant colif. bact. (MF) m-FC Agar, 44 °C according to SS 028167	5.75 <sup>3</sup>	0.12	0.37	2.84	0.00	0.71	4.26 <sup>4</sup>	0.08	0.44
<i>Escherichia coli</i> (MF) SS-EN ISO 9308-1:2014	3.19 <sup>2</sup>	0.33	<b>0.03</b>	3.88	0.31	0.09	-	-	-
Intestinal enterococci (MF) SS-EN ISO 7899-2:2000	6.38 <sup>4</sup>	0.00	0.57	6.60 <sup>5</sup>	0.36	<b>0.01</b>	-	-	-
<i>Pseudomonas aeruginosa</i> (MF) SS-EN ISO 16288:2008	-	-	-	5.52 <sup>5</sup>	0.00	0.99	3.38 <sup>4</sup>	0.15	0.41
Culturable microorg., 48 h 37 °C (pour plate) SS-EN ISO 6222:1999	5.62	0.39	<b>0.01</b>	7.93	0.00	0.58	6.05	0.28	0.23
Culturable microorg., 72 h 22 °C (pour plate) SS-EN ISO 6222:1999	5.26	0.30	0.24	1.61	0.00	0.79	6.48	0.20	0.30

– No target organism or no value

<sup>1</sup> n = 5 vials analysed in duplicate

<sup>2</sup> cfu per 1 ml of sample

<sup>3</sup> cfu per 5 ml of sample

<sup>4</sup> cfu per 10 ml of sample

<sup>5</sup> cfu per 50 ml of sample

# References

1. ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Ilbäck J and Blom L. 2024. Protocol – Microbiological Proficiency Testing, Swedish Food Agency.
3. Peterz, M., 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



Appendix 1. Results of the participating laboratories

Lab no.	Coliform bacteria			Suspected thermotolerant coliform bacteria			E. coli			Intestinal enterococci			P. aeruginosa			Culturable microorganisms, 22 °C for 3 days (cfu/ml)			Culturable microorganisms, 37 °C for 2 days (cfu/ml)			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
6456-1	2045	11	427	-	-	-	773	11	0	382	67	0	-	-	-	29	-	45	33	55	35	6456-1
6456-2	1500	14	336	-	-	-	718	4	0	-	-	-	-	-	-	0	-	-	-	-	-	6456-2
6686-1	831	9.9	31	-	-	-	324	9.9	0	290	77	0	-	-	-	17	2	41	24	68	42	6686-1
6686-2	1100	1	200	-	-	-	570	1	0	-	-	-	-	-	-	-	-	-	-	-	-	6686-2
7248-1	2500	40	220	900	11	80	1000	40	0	310	130	0	0	49	80	16	5	35	24	53	41	7248-1
7248-2	2282	21	384	-	-	-	810	11	0	-	-	-	-	-	-	-	-	-	-	-	-	7248-2
7330-1	-	-	-	-	-	-	300	2	0	307	88	21	0	32	68	-	-	-	69	100	83	7330-1
7330-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7330-2
7381-1	1210	30	450	-	-	-	-	-	-	-	-	-	130	37	69	13	3	34	30	49	32	7381-1
7381-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7381-2
7442-1	1673	37	168	-	-	-	823	19	0	386	91	0	-	-	-	21	2	41	-	-	-	7442-1
7442-2	1468	30	321	-	-	-	645	20	0	-	-	-	-	-	-	-	-	-	-	-	-	7442-2
7564-1	1600	25	420	-	-	-	750	9	0	-	-	-	-	-	-	30	10	41	49	510	36	7564-1
7564-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7564-2
7688-1	1300	6	276	-	-	-	727	6	0	276	64	0	0	33	93	25	3	31	33	70	44	7688-1
7688-2	-	-	-	-	-	-	-	-	-	430	29	0	0	43	109	-	-	-	-	-	-	7688-2
7728-1	1800	15	200	-	-	-	830	15	0	470	4	0	0	38	71	25	1	34	-	63	31	7728-1
7728-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27	-	-	7728-2
7876-1	1590	38	260	170	0	30	720	8	0	310	71	0	0	62	120	25	2	41	28	56	40	7876-1
7876-2	1986	28	540	-	-	-	816	28	0	-	-	-	-	-	-	-	-	-	-	-	-	7876-2
7930-1	2200	13	480	-	-	-	1000	13	0	380	82	0	0	41	95	32	4	38	23	50	43	7930-1
7930-2	1700	25	340	-	-	-	780	25	0	-	-	-	-	-	-	-	-	-	-	-	-	7930-2
7962-1	2420	16	162	610	5	38	1046	16	0	435	74	0	0	37	62	27	3	33	22	67	32	7962-1
7962-2	2300	33	370	-	-	-	1100	11	0	430	91	0	-	-	-	-	-	-	-	-	-	7962-2
8068-1	1376	23	323	-	-	-	309	7	0	300	90	0	0	60	51	28	1	35	25	81	44	8068-1
8068-2	-	-	-	-	-	-	-	-	-	365	86	0	-	-	-	-	-	-	-	-	-	8068-2
8165-1	1509	38	350	-	-	-	800	15	0	345	83	0	0	54	122	-	-	-	-	-	-	8165-1
8165-2	1586	49	320	-	-	-	958	20	0	532	68	0	-	-	-	-	-	-	-	-	-	8165-2
8260-1	1468	33	20	-	-	-	714	9	0	-	-	-	-	-	-	26	2	35	-	-	-	8260-1
8260-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8260-2
8329-1	1733	30	460	-	-	-	1046	17	0	465	26	0	0	48	57	27	1	52	17	59	32	8329-1
8329-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8329-2
8380-1	1400	14	339	-	-	-	570	14	0	315	88	0	0	34	87	18	2	38	20	60	42	8380-1
8380-2	1100	34	250	-	-	-	550	13	0	-	-	-	0	33	52	-	-	-	-	-	-	8380-2
8435-1	1510	34	0	-	-	-	690	14	0	260	79	0	-	-	-	16	1	33	-	-	-	8435-1
8435-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8435-2
8569-1	1860	59	498	-	-	-	860	24	0	352	-	-	-	-	-	31	-	-	-	-	-	8569-1
8569-2	1565	46	461	-	-	-	884	31	0	-	46	0	-	-	-	1	46	-	-	-	-	8569-2
8626-1	2400	12	592	900	10	0	1920	12	0	350	0	0	-	-	-	31	0	27	25	48	42	8626-1
8626-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8626-2
8628-1	1730	36	180	720	13	310	810	19	0	270	61	0	0	63	80	26	2	48	21	79	41	8628-1
8628-2	-	-	-	-	-	-	720	13	0	-	-	-	-	-	-	-	-	-	-	-	-	8628-2
8663-1	2100	31	600	1500	21	450	1100	16	0	350	78	0	0	37	61	31	5	36	19	75	41	8663-1
8663-2	1700	16	440	-	-	-	530	16	0	390	56	0	0	40	68	-	-	-	-	-	-	8663-2
8742-1	1500	15	140	-	-	-	800	15	0	-	-	-	-	-	-	31	1	35	31	60	-	8742-1
8742-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	8742-2
8751-1	1500	14	59	-	-	-	1110	14	0	324	66	0	-	-	-	27	5	41	-	-	-	8751-1
8751-2	-	22	-	-	-	-	-	22	-	-	-	-	-	-	-	-	-	-	-	-	-	8751-2
8766-1	1490	23	110	654	0	78	709	13	0	354	60	0	0	56	80	24	2	27	19	18	32	8766-1
8766-2	1744	24	266	-	-	-	760	0	0	-	-	-	-	-	-	-	-	-	-	-	-	8766-2
8862-1	1854	41	209	-	-	-	845	16	0	290	47	0	0	85	102	27	0	40	36	81	31	8862-1
8862-2	2007	57	480	-	-	-	1204	25	0	418	83	0	-	-	-	-	-	-	-	-	-	8862-2
8891-1	300	0	120	-	-	-	-	-	-	-	-	-	-	-	-	15	3	31	-	-	-	8891-1
8891-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8891-2
9002-1	870	57	3600	-	-	-	810	13	0	370	74	0	-	-	-	25	0	46	-	-	-	9002-1
9002-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9002-2
9306-1	1733	15	291	-	-	-	921	15	0	-	-	-	-	-	-	27	3	27	26	74	51	9306-1
9306-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9306-2
9408-1	1733	19	345	-	-	-	921	19	0	230	47	0	0	51	850	26	2	34	31	55	41	9408-1
9408-2	1410	19	240	-	-	-	690	5	0	350	8	0	-	-	-	24	1	27	13	50	35	9408-2
9436-1	1900	36	455	736	6	209	1000	17	0	255	87	0	-	-	-	32	3	34	23	75	30	9436-1
9436-2	1953	19	208	-	-	-	1086	19	0	-	-	-	-	-	-	-	-	-	-	-	-	9436-2
9524-1	2300	34	330	-	-	-	1027	17	0	300	79	0	-	-	-	32	7	40	45	74	55	9524-1
9524-2	1723	16	345	-	-	-	1081	16	0	393	56	0	-	-	-	-	-	-	-	-	-	9524-2
9736-1	1727	22	342	-	-	-	818	6	0	410	61	0	0	63	92	26	3	42	29	70	32	9736-1
9736-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9736-2
9857-1	1553	40	461	-	-	-	770	23	0	276	68	0	-	-	-	27	4	34	-	-	-	9857-1
9857-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9857-2
9899-1	1964	16	332	-	-	-	1082	16	0	330	75	0	0	46	102	35	2	45	36	62	45	9899-1
9899-2	2059	25	366	-	-	-	1018	17	0	353	71	0	0	44	98	-	-	-	-	-	-	9899-2
9903-1	1032	12	358	-	-	-	740	12	0	413	99	0	0	61	126	34	2	37	-	63	40	9903-1
9903-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33	-	-	9903-2
N	106	107	106	18	18	18	107	108	107	82	83	83	53	54	54	75	75	75	57	57	57	N
n	99	101	98	18	18	18	100	100	106	82	82	82	50	51	51	71	73	74	54	50	56	n
m <sub>PT</sub>	40.83	4.90	17.81	-	-	-	28.68	3.81	-	18.87	8.38	-	-	6.77	9.21	5.10	1.37	6.11	5.11	7.78	6.19	m <sub>PT</sub>
s <sub>PT</sub>	5.54	1.22	4.69	-	-	-	3.85	0.76	-	1.86	1.14	-	-	0.98	1.62	0.55	0.59	0.51	0.60	0.93	0.50	s <sub>PT</sub>
u <sub>PT</sub>	0.675	0.151	0.583	-	-	-	0.472	0.093	-	0.257	0.157	-	-	0.168	0.276	0.079	0.085	0.073	0.100	0.156	0.083	u <sub>PT</sub>
CV (%)	14	25	2																			



## Appendix 1. Results of the participating laboratories

Lab no.	Coliform bacteria			Suspected thermotolerant coliform bacteria			E. coli			Intestinal enterococci			P. aeruginosa			Culturable microorganisms, 22 °C for 3 days (cfu/ml)			Culturable microorganisms, 37 °C for 2 days (cfu/ml)			Lab no.
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	

N = number of reported results  
 n = results without annotation  
 < = low outlier  
 > = high outlier

Min = lowest reported result  
 Max = highest reported result  
 Lower = lowest accepted value  
 Upper = highest accepted value

Med = median value  
 m<sub>PT</sub> = assigned value  
 F+ = false positive  
 F- = false negative

s<sub>PT</sub> = standard deviation  
 u<sub>PT</sub> = measurement uncertainty  
 CV = coefficient of variation  
 u<sub>rel,mPT</sub> = relative standard uncertainty of m<sub>PT</sub>

- 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<sub>PT</sub> > 0,3 s<sub>PT</sub> and/or > 20 % outliers and/or fewer than 12 evaluated results
- Result** The result is excluded prior determining m<sub>PT</sub> and s<sub>PT</sub>



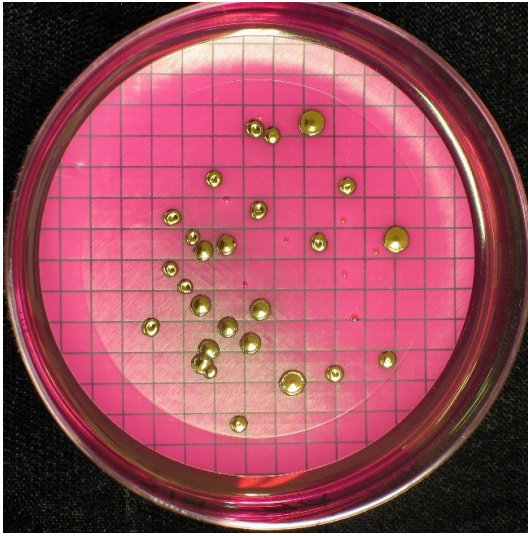
## Appendix 2. Z-scores of all participants

Lab no.	Coliform bacteria			Suspected thermotolerant coliform bacteria			E. coli			Intestinal enterococci			P. aeruginosa			Culturable microorganisms, 22°C for 3 days			Culturable microorganisms, 37 °C for 2 days			Lab no.	
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
6456-1	0.794	-1.293	0.608				-0.227	-0.648	0	0.363	-0.171	0				0.523		1.187	1.054	-0.387	-0.541	6456-1	
6456-2	-0.379	-0.946	0.111				-0.488	-2.377	0								-2.342						6456-2
6686-1	-2.167	-1.432	-2.610				-2.773	-0.872	0	-0.990	0.348	0				-1.782	0.073	0.585	-0.359	0.505	0.589	6686-1	
6686-2	-1.383	-3.188	-0.782				-1.247	-3.690	0														6686-2
7248-1	1.657	1.166	-0.635				0.765	3.301	0	-0.680	2.661	0	0	0.233	-0.162	-2.007	1.476	-0.377	-0.359	-0.533	0.433	7248-1	
7248-2	1.254	-0.258	0.381				-0.056	-0.648	0														7248-2
7330-1							-2.949	-3.146	0	-0.725	0.882		0	-1.139	-0.592				4.000	2.388	4.000	7330-1	
7330-2																							7330-2
7381-1	-1.091	0.474	0.725											-0.704	-0.554	-2.727	0.615	-0.545	0.607	-0.833	-1.060	7381-1	
7381-2																							7381-2
7442-1	0.014	0.969	-1.034				0.003	0.720	0	0.418	1.022	0				-0.943	0.073	0.585				7442-1	
7442-2	-0.454	0.474	0.023				-0.852	0.869	0														7442-2
7564-1	-0.149	0.083	0.572				-0.335	-1.064	0							0.691	3.057	0.585	3.150	4.000	-0.373	7564-1	
7564-2																							7564-2
7688-1	-0.862	-2.002	-0.255				-0.445	-1.787	0	-1.214	-0.334	0	0	-1.050	0.269	-0.181	0.615	-1.064	1.054	0.634	0.894	7688-1	
7688-2							-0.194	1.562	0	1.004	-2.636	0	0	-0.219	0.759								7688-2
7728-1	0.289	-0.838	-0.782				0.035	0.082	0	1.511	-4.000	0	0	-0.621	-0.481	-0.181	-0.635	-0.545		0.173	-1.238	7728-1	
7728-2																				0.138			7728-2
7876-1	-0.172	1.036	-0.359				-0.479	-1.289	0	-0.680	0.041	0	0	1.127	1.076	-0.181	0.073	0.585	0.297	-0.314	0.276	7876-1	
7876-2	0.675	0.322	1.157				-0.029	1.944	0														7876-2
7930-1	1.098	-1.057	0.874				0.765	-0.269	0	0.335	0.595	0	0	-0.377	0.332	1.019	1.073	0.114	-0.531	-0.757	0.742	7930-1	
7930-2	0.073	0.083	0.134				-0.194	1.562	0														7930-2
7962-1	1.511	-0.734	-1.083				0.952	0.249	0	1.069	0.196	0	0	-0.704	-0.821	0.177	0.615	-0.715	-0.707	0.439	-1.060	7962-1	
7962-2	1.288	0.692	0.304				1.166	-0.648	0	1.004	1.022	0											7962-2
8068-1	-0.674	-0.084	0.035				-2.882	-1.529	0	-0.834	0.975	0	0	0.996	-1.272	0.351	-0.635	-0.377	-0.190	1.314	0.894	8068-1	
8068-2										0.126	0.788	0											8068-2
8165-1	-0.358	1.036	0.191				-0.102	0.082	0	-0.159	0.644	0	0	0.590	1.132							8165-1	
8165-2	-0.181	1.719	0.017				0.591	0.869	0	2.257	-0.117	0											8165-2
8260-1	-0.454	0.692	-2.843				-0.508	-1.064	0							0.000	0.073	-0.377				8260-1	
8260-2																							8260-2
8329-1	0.145	0.474	0.775				0.952	0.410	0	1.449	-2.888	0	0	0.160	-1.020	0.177	-0.635	2.180	-1.655	-0.102	-1.060	8329-1	
8329-2																							8329-2
8380-1	-0.616	-0.946	0.128				-1.247	-0.090	0	-0.603	0.882	0	0	-0.961	0.074	-1.564	0.073	0.114	-1.072	-0.032	0.589	8380-1	
8380-2	-1.383	0.763	-0.426				-1.357	-0.269	0				0	-1.050	-1.229								8380-2
8435-1	-0.355	0.763					-0.625	-0.090	0	-1.477	0.448	0				-2.007	-0.635	-0.715				8435-1	
8435-2																							8435-2
8569-1	0.416	2.276	0.961				0.169	1.429	0	-0.058						0.856						8569-1	
8569-2	-0.229	1.541	0.780				0.274	2.307	0		-1.406	0					-0.635	1.333				8569-2	
8626-1	1.475	-1.173	1.390				3.933	-0.455	0	-0.087		0				0.856	-2.342	-1.798	-0.190	-0.911	0.589	8626-1	
8626-2																							8626-2
8628-1	0.139	0.901	-0.937				-0.056	0.720	0	-1.312	-0.501	0	0	1.191	-0.162	0.000	0.073	1.621	-0.887	1.194	0.433	8628-1	
8628-2							-0.479	-0.269	0														8628-2
8663-1	0.903	0.548	1.425				1.166	0.249	0	-0.087	0.398	0	0	-0.704	-0.860	0.856	1.476	-0.211	-1.261	0.949	0.433	8663-1	
8663-2	0.073	-0.734	0.675				-1.468	0.249	0	0.473	-0.789	0	0	-0.457	-0.592							8663-2	
8742-1	-0.379	-0.838	-1.274				-0.102	0.082	0							0.856	-0.635	-0.377	0.758	-0.032		8742-1	
8742-2																					0.276		8742-2
8751-1	-0.379	-0.946	-2.159				1.205	-0.090	0	-0.468	-0.225	0				0.177	1.476	0.585				8751-1	
8751-2								1.155															8751-2
8766-1	-0.402	-0.084	-1.561				-0.532	-0.269	0	-0.030	-0.558	0	0	0.727	-0.162	-0.365	0.073	-1.798	-1.261	-3.794	-1.060	8766-1	
8766-2	0.169	0.001	-0.320				-0.288		0														8766-2
8862-1	0.403	1.231	-0.715				0.102	0.249	0	-0.990	-1.342	0	0	2.502	0.550	0.177	-2.342	0.430	1.480	1.314	-1.238	8862-1	
8862-2	0.718	2.168	0.874				1.564	1.562	0	0.847	0.644	0											8862-2
8891-1	-4.000		-1.462													-2.239	0.615	-1.064				8891-1	
8891-2																							8891-2
9002-1	-2.046	2.168	4.000				-0.056	-0.269	0	0.197	0.196	0				-0.181	-2.342	1.333				9002-1	
9002-2																							9002-2
9306-1	0.145	-0.838	-0.160				0.434	0.082	0							0.177	0.615	-1.798	-0.025	0.887	1.911	9306-1	
9306-2																							9306-2
9408-1	0.145	-0.441	0.163				0.434	0.720	0	-1.993	-1.342	0	0	0.378	4.000	0.000	0.073	-0.545	0.758	-0.387	0.433	9408-1	
9408-2	-0.592	-0.441	-0.494				-0.625	-2.067	0	-0.087	-4.000	0				-0.365	-0.635	-1.798	-2.519	-0.757	-0.541	9408-2	
9436-1	0.499	0.901	0.751				0.765	0.410	0	-1.561	0.835	0		0.378	1.270	1.019	0.615	-0.545	-0.531	0.949	-1.419	9436-1	
9436-2	0.608	-0.441	-0.722				1.111	0.720	0														9436-2
9524-1	1.288	0.763	0.076				0.875	0.410	0	-0.834	0.448	0				1.019	2.175	0.430	2.663	0.887	2.460	9524-1	
9524-2	0.123	-0.734	0.163				1.091	0.249	0	0.513	-0.789	0										9524-2	
9736-1	0.132	-0.170	0.146				-0.020	-1.787	0	0.742	-0.501	0	0	1.191	0.237	0.000	0.615	0.738	0.453	0.634	-1.060	9736-1	
9736-2																							9736-2
9857-1	-0.256	1.166	0.780				-0.241	1.294	0	-1.214	-0.117	0				0.177	1.073	-0.545				9857-1	
9857-2																							9857-2
9899-1	0.631	-0.734	0.088				1.095	0.249	0	-0.379	0.247	0	0	0.011	0.550	1.492	0.073	1.187	1.480	0.105	1.044	9899-1	
9899-2	0.822	0.083	0.282				0.839	0.410	0	-0.044	0.041	0	0	-0.141	0.426							9899-2	
9903-1	-1.572	-1.173	0.237				-0.383	-0.455	0	0.781	1.383	0	0	1.062	1.242	1.337	0.073	-0.047		0.173	0.276	9903-1	
9903-2																			1.054				9903-2

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

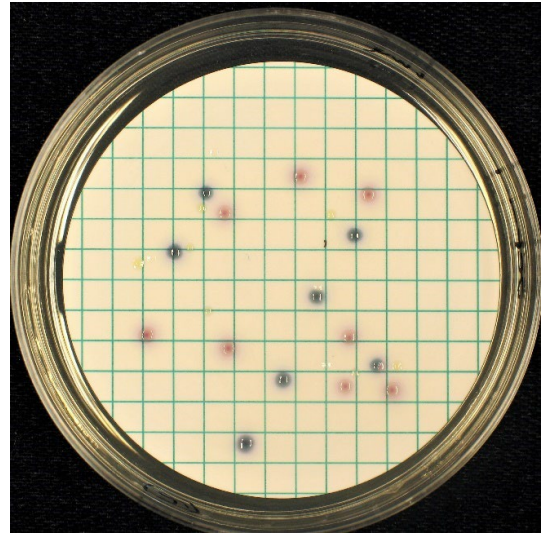
Sample A

m-Endo Agar LES, 37 °C



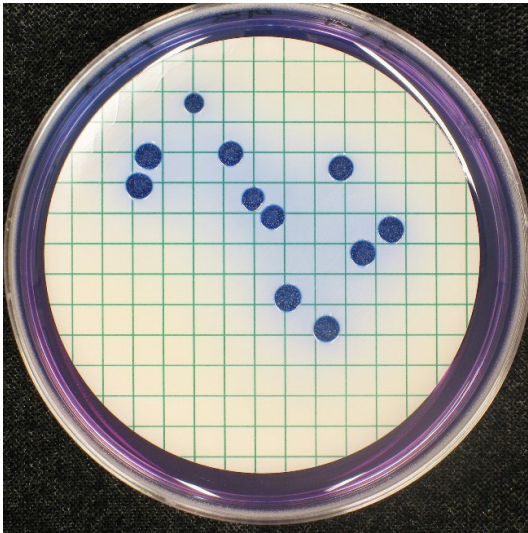
1 ml

Chromocult Coliform Agar, 37 °C



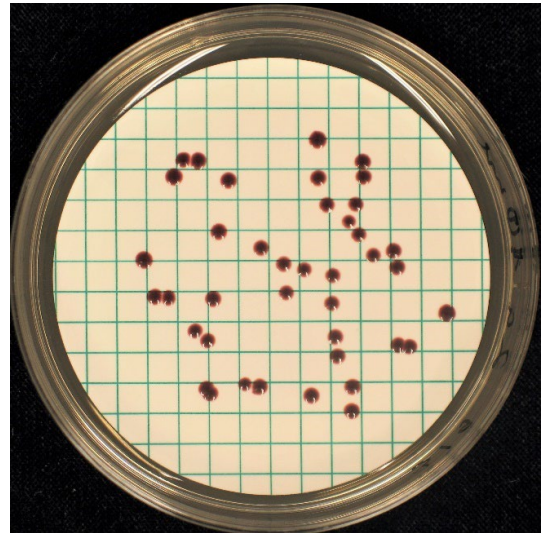
1 ml

m-FC Agar, 44 °C



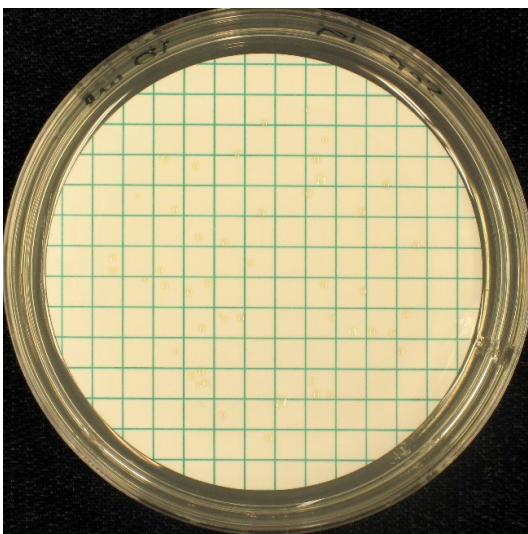
1 ml

m-Enterococcus Agar, 37 °C



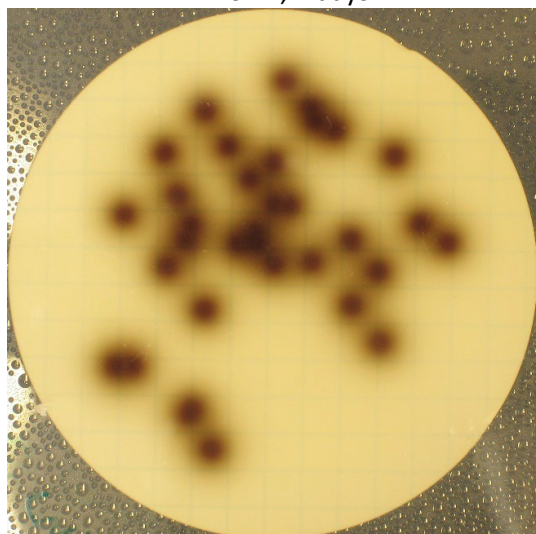
10 ml, 2 days

m-Pseudomonas CN Agar, 37 °C



10 ml, 2 days

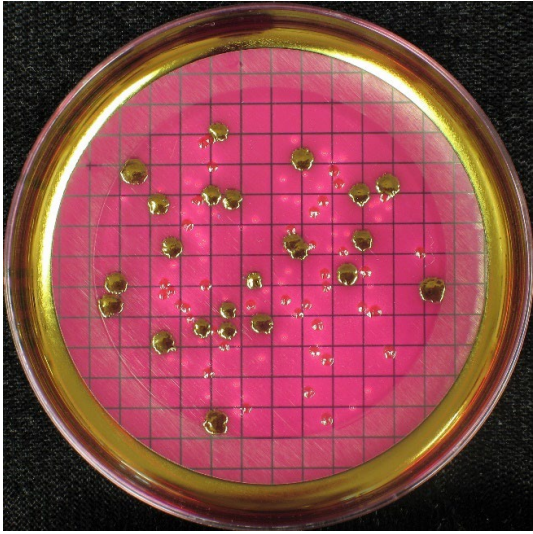
Bile Esculin Azide Agar, 44 °C



10 ml, 2 hours (from beneath)

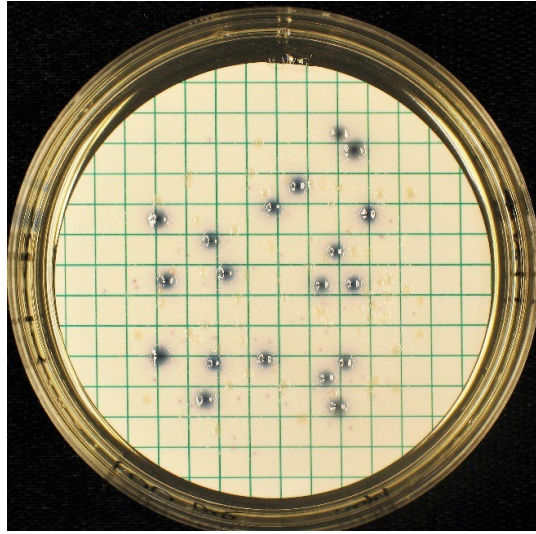
Sample B

m-Endo Agar LES, 37 °C



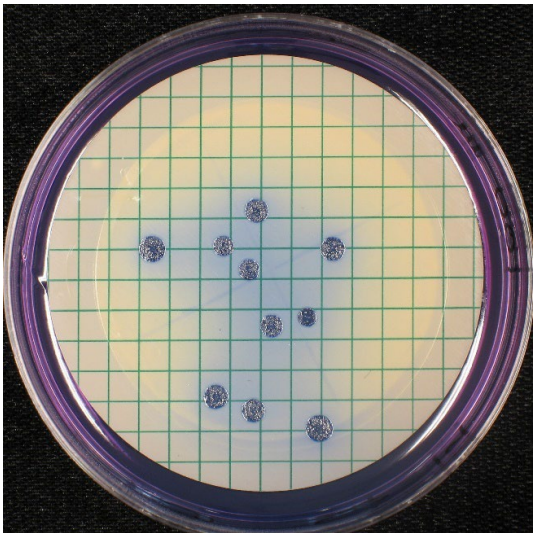
100 ml

Chromocult Coliform Agar, 37 °C



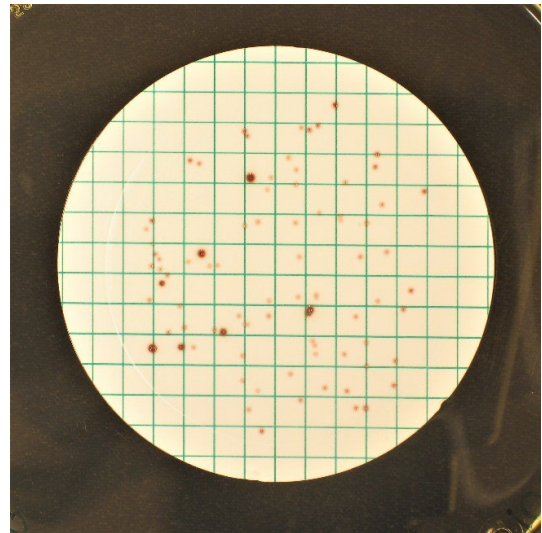
100 ml

m-FC Agar, 44 °C



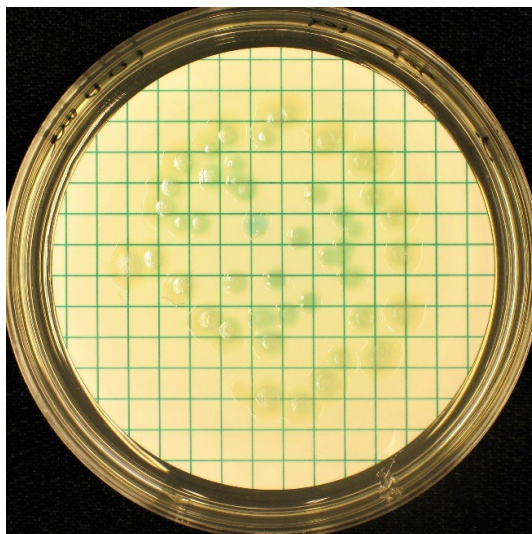
100 ml

m-Enterococcus Agar, 37 °C



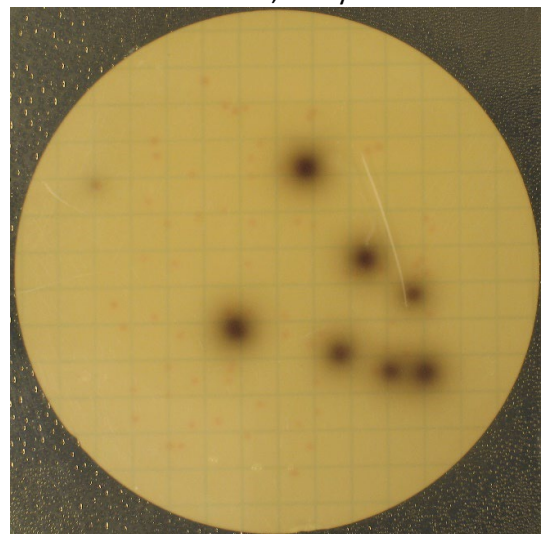
100 ml, 2 days

m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

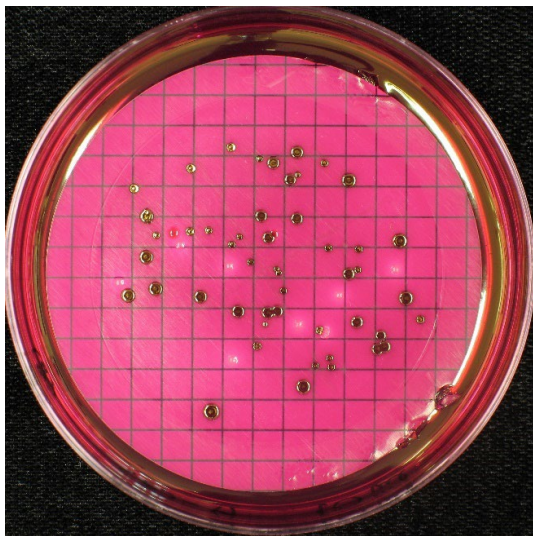
Bile Esculin Azide Agar, 44 °C



100 ml, 2 hours (from beneath)

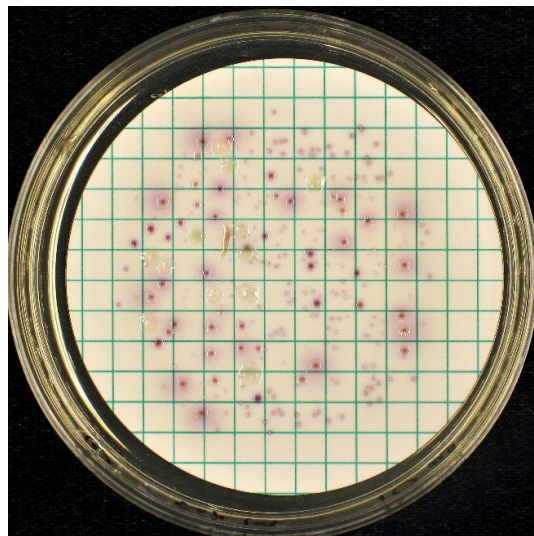
Sample C

m-Endo Agar LES, 37 °C



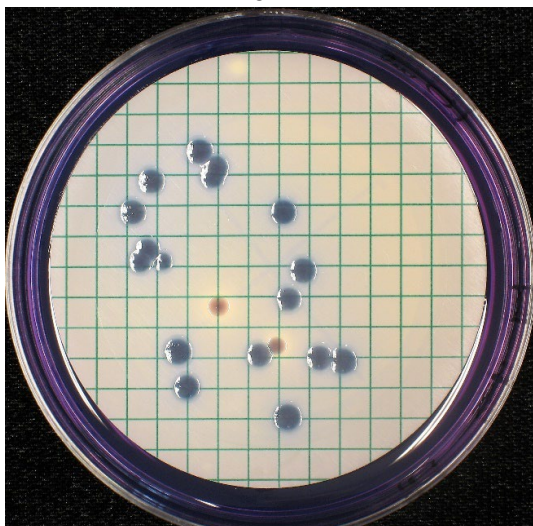
10 ml

Chromocult Coliform Agar, 37 °C



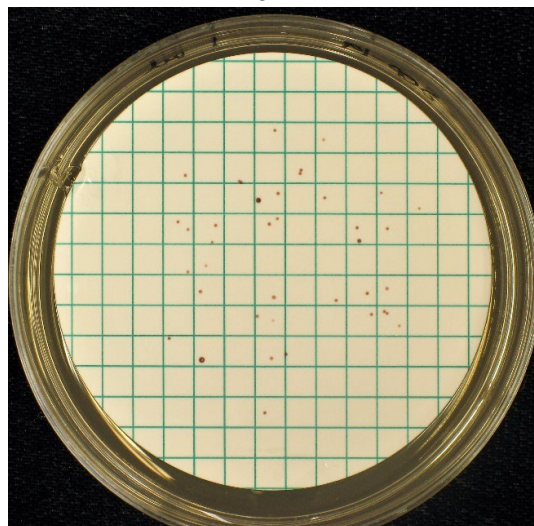
10 ml

m-FC Agar, 44 °C



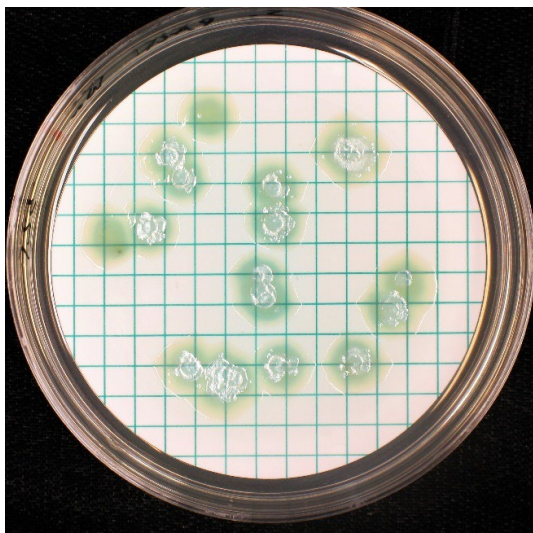
10 ml

m-Enterococcus Agar, 37 °C



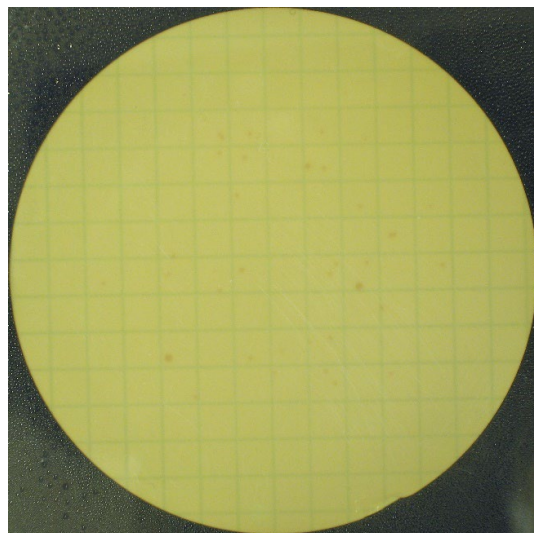
1 ml

m-Pseudomonas CN Agar, 37



10 ml, 2 days

Bile Esculin Azide Agar, 44 °C



1 ml, 2 days (from beneath)

## Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most participants carry out some form of internal quality assurance, but the analytical work also needs to be evaluated by an independent party. Such external quality control of laboratory competence is commonly required by accreditation bodies and can be done by taking part in proficiency testing (PT).

In a PT, identical test material is analysed by a number of participants. After reporting of results by the participants, the organiser evaluates the results and compiles them in a report.

### The Swedish Food Agency's PT program offers

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

For more information, visit our website: [www.livsmedelsverket.se/en/PT-micro](http://www.livsmedelsverket.se/en/PT-micro)

### The Swedish Food Agency's reference material

As a complement to the proficiency testing, but without specific accreditation, the Swedish Food Agency also manufactures a number of reference materials (RM) for internal quality control of food and drinking water microbiological analyses, including pathogens.

For more information, visit our website: [www.livsmedelsverket.se/en/RM-micro](http://www.livsmedelsverket.se/en/RM-micro)