

## Drinking Water Microbiology

September 2019

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*Edition*  
Version 1 (2019-11-27)

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PT September 2019 is registered as no. 2019/02619 at the Swedish Food Agency, Uppsala

*Proficiency testing*  
**Drinking water Microbiology**  
September 2019

**Parameters included**

**Coliform bacteria** and *Escherichia coli* with membrane filter method (MF)

**Coliform bacteria** and *Escherichia coli*, (rapid methods with MPN)

**Suspected thermotolerant coliform bacteria** with MF (not assessed)

**Intestinal enterococci** with MF/MPN

*Pseudomonas aeruginosa* with MF/MPN

**Culturable microorganisms** (total count) 3 days incubation at  $22\pm 2$  °C

**Culturable microorganisms** (total count) 2 days incubation at  $36\pm 2$  °C

## Abbreviations and explanations




### *Microbiological media*

CCA	Chromocult Coliform Agar <sup>®</sup> (Merck; EN ISO 9308-1:2014)
Colilert	Colilert <sup>®</sup> Quanti-Tray <sup>®</sup> (IDEXX Inc.; EN ISO 9308-2:2014)
Enterolert	Enterolert <sup>®</sup> Quanti-Tray <sup>®</sup> (IDEXX Inc.)
LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (according to EN ISO 9308-1:2000)
m-Ent	m-Enterococcus Agar (Slanetz & Bartley; accord. to EN ISO 7899-2:2000)
m-FC	m-FC Agar (according to SS 028167)
PACN	Pseudomonas Agar base/CN agar (with cetrimide and nalidixic acid; according to EN ISO 16266:2008)
Pseudalert	Pseudalert <sup>®</sup> Quanti-Tray <sup>®</sup> (IDEXX Inc.; ISO 16266-2:2018)
YEA	Yeast extract Agar (according to EN ISO 6222:1999)




### *Other abbreviations*

MF	Membrane filter (method)
MPN	"Most Probable Number" (quantification based on statistical distributions)
ISO	"International Organization for Standardization" and their standards
EN	European standard from "Comité Européen de Normalisation" (CEN)
NMKL	"Nordisk Metodikkomité for næringsmidler" and their standards
DS, NS, SFS, SS	National standards from Denmark, Norway, Finland and Sweden

### *Legend to method comparison tables*

N	total number of laboratories that reported methods and numerical results
n	number of results except false results and outliers
Mv	mean value (with outliers and false results <i>excluded</i> )
Med	median value (with outliers and false results <i>included</i> )
CV	coefficient of variation = relative standard deviation in percentage of the mean, calculated from square root transformed results
F	number of false positive or false negative results
<	number of low outliers
>	number of high outliers
	total number of results for the parameter
	remarkably low result
	remarkably high result or CV or many deviating results

### *Explanations to histograms with accepted and deviating results*

	result without remark
	false negative result
	outlier
↓ 34	average without deviating results
*	over a bar means that the result is beyond the x-axis limit

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## General information on results evaluation

The proficiency testing program organised by the Swedish Food Agency is accredited against EN ISO/IEC 17043. This standard prescribes that results should be grouped based on the methods used. Therefore it is mandatory for participants to inform about method data. This report presents, for each parameter, method data where differences are present or could be expected.

The method information gathered is sometimes difficult to interpret. Sometimes there is inconsistency between the standard referred to and the information regarding various method details. Results from laboratories with ambiguous details are either excluded or placed in the group "Other/Unknown" in the tables, together with results from methods used only by individual laboratories. Thus, to get an as appropriate evaluation as possible of the results, it is important that correct standards and method details are reported.

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in tables together with the group means etc. The mean and measure of dispersion are not shown for groups with four or fewer results, other than exceptionally when it is specifically mentioned. However, all results are shown in the method histogram when possible.

The histograms and calculation of outliers are described on page 29 under "Processing of numerical results" with further reference to the scheme protocol [1].

## Results of the PT round

### General outcome

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Test items were sent to 94 laboratories, 34 in Sweden, 52 in other Nordic countries (Faeroe Islands, Greenland and Åland included), 3 more from EU, 1 from the rest of Europe and 4 from outside Europe. Results were reported from 90 laboratories.

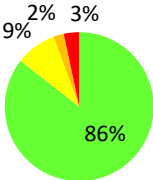
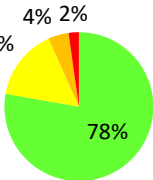
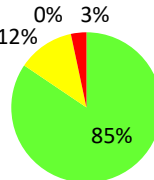
The percentages of false results and outliers are compiled in **table 1**.

Microorganisms and parameters of analyses are also compiled in table 1. For the MF analyses the parameters *suspected* coliform and thermotolerant coliform bacteria could be reported (shaded column in table 1 and table 3), as well as *suspected* intestinal enterococci and *suspected* *Pseudomonas aeruginosa* on primary media. The results from suspected colonies are only used for interpretations and discussions, not for assessment.

All reported results are compiled in **annex A** and results for each laboratory are also shown on our website after logging in (<https://www2.slv.se/absint/>).

Standardized z-scores for all evaluated results are given in **annex B** and photographs with examples of colony appearance on various media are presented in **annex C**.

**Table 1** Microorganisms in each sample and percentages of deviating results (F%: false positive or false negative, X%: outliers); parameters with grey shading are not assessed

Sample	A			B			C		
Percentage of laboratories with									
No. of evaluable results	544			541			547		
No. of deviating results *	22 (4 %)			31 (6 %)			23 (4 %)		
Microorganisms	<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 hydrophila</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus saprophyticus</i>		
Analysis	Target org.	F%	X%	Target org.	F%	X%	Target org.	F%	X%
Coliform bacteria (MF)	<i>E. coli</i> <i>E. cloacae</i>	0	1	<i>E. coli</i> { <i>H. alvei</i> }	3	3	<i>C. sakazakii</i> [ <i>A. hydrophila</i> ]	10	0
Susp. thermotolerant coliform bact. (MF)	<i>E. coli</i> { <i>E. cloacae</i> }	–	–	<i>E. coli</i>	–	–	<i>C. sakazakii</i>	–	–
<i>E. coli</i> (MF)	<i>E. coli</i>	0	7	<i>E. coli</i>	8	0	–	1	–
Coliform bacteria (rapid method)	<i>E. coli</i> <i>E. cloacae</i>	0	2	<i>E. coli</i> <i>H. alvei</i>	0	2	<i>C. sakazakii</i>	2	2
<i>E. coli</i> (rapid meth.)	<i>E. coli</i>	2	2	<i>E. coli</i>	3	0	–	2	0
Intestinala enterokocker (MF)	<i>E. faecalis</i>	0	3	<i>E. faecium</i>	11	0	[ <i>S. saprophyticus</i> ]	6	–
<i>Pseudomonas aeruginosa</i> (MF)	[ <i>B. cepacia</i> ]	7	0	<i>P. aeruginosa</i>	2	3	<i>P. aeruginosa</i>	0	2
Culturable micro-organisms (total count), 3 days 22 °C	<i>B. cepacia</i> <i>E. coli</i> <i>E. cloacae</i> <i>E. faecalis</i>	0	5	<i>E. faecium</i> <i>E. coli</i> <i>H. alvei</i> <i>P. aeruginosa</i>	0	7	<i>S. saprophyticus</i> <i>A. hydrophila</i> <i>C. sakazakii</i> <i>P. aeruginosa</i>	0	6
Culturable micro-organisms (total count), 2 days 36 °C	<i>B. cepacia</i> <i>E. coli</i> <i>E. cloacae</i> <i>E. faecalis</i>	0	4	<i>S. capitis</i> <i>E. faecium</i> <i>E. coli</i> <i>H. alvei</i> <i>P. aeruginosa</i>	0	3	<i>S. saprophyticus</i> <i>A. hydrophila</i> <i>C. sakazakii</i> <i>P. aeruginosa</i>	0	3

\* In total 32 of 90 laboratories (36 %) reported at least one deviating result

– Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

[ ] The organism may be presumptively false positive on the primary growth medium

{ } The organism may give different results depending on method or definition used

## Coliform bacteria (MF)

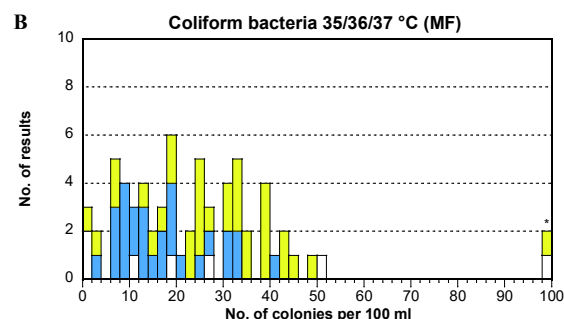
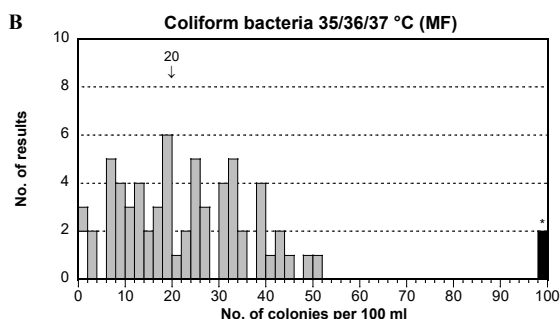
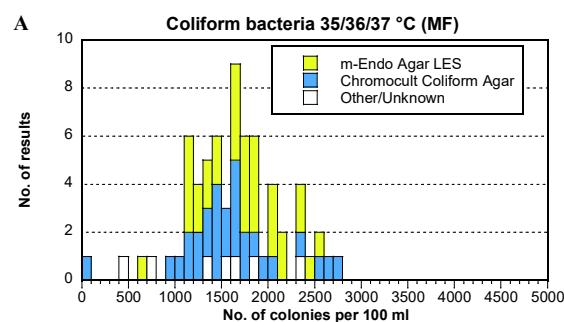
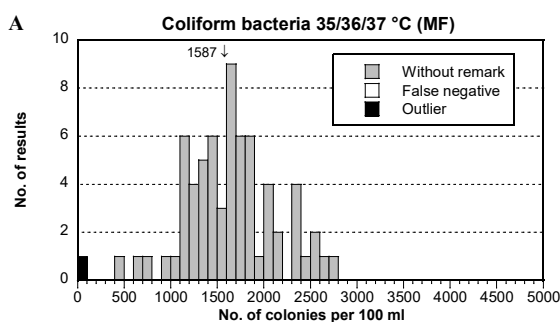
In the group Other/Unknown in the table six different media are used, based on methods for both water and food, as well as for the medical field.

From the table it is clear that approximately the same number of laboratories used CCA and LES. The proportion that used CCA is no longer increasing in relation to LES, as has been seen since the standard EN ISO 8308-1 from 2014 was issued. The use of LTTC for coliform bacteria has ceased.

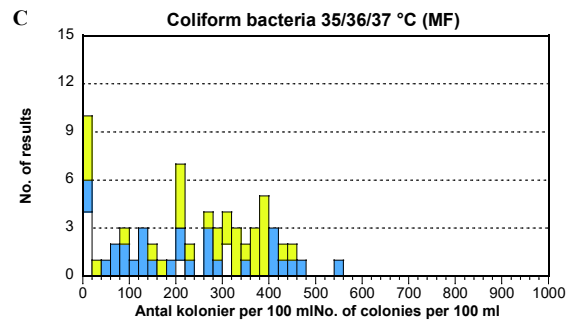
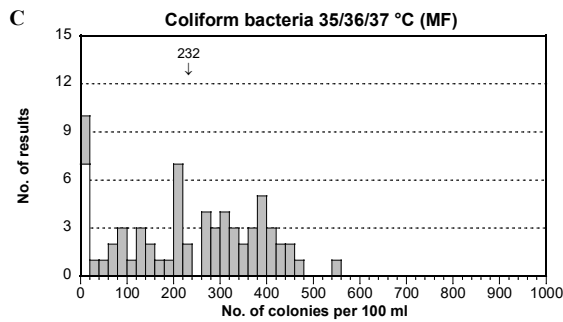
The average results for LES and CCA are approximately equal only in sample A. In both sample B and C the results are lower for CCA, as has also often been the case previously. As the media are based on different standards, the differences apply also to these standards. The heterogenic group Other/Unknown contained several low results. In sample A this group had a lower average than other groups while it contained several false negative results in sample B and C.

In total five coliform bacteria, including *E. coli*, were present in the samples.

Medium	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	67	66	<b>1587</b>	15	0	0	0	62	<b>20</b>	32	2	0	2	60	<b>232</b>	33	7	0	0
m-Endo Agar LES	32	32	<b>1645</b>	13	0	0	0	31	<b>24</b>	29	0	0	1	30	<b>251</b>	31	2	0	0
Chromocult C Agar	28	27	<b>1599</b>	14	0	1	0	27	<b>15</b>	31	0	0	0	27	<b>207</b>	37	1	0	0
Other/Unknown	7	7	<b>1292</b>	27	0	0	0	4	–	–	2	0	1	3	–	–	<b>4</b>	0	0







### Sample A

- A strain of *Escherichia coli* and a strain of *Enterobacter cloacae* were included. They appeared with for coliform bacteria typical colonies on the MF media at 37 °C, a metallic sheen on LES and blue and pinkish red, respectively, on CCA.
- The distribution of the results was good with small dispersion (CV; see page 29). One low outlier was the only deviating result.

### Sample B

- One strain each of *E. coli* and *Hafnia alvei* were present as coliform bacteria. *E. coli* appeared at the Swedish Food Agency with for coliform bacteria typical colonies on the MF media at 37 °C, a typical metallic sheen on LES and blue on CCA. The colonies of *H. alvei* were red without metallic sheen on LES and light beige to pink or pale apricot coloured on CCA. This means that *H. alvei* could be included as a coliform bacterium on CCA but not on LES. The results, however, indicate the opposite, as they are lower for CCA. The colonies that have been counted from the two media seem to vary among the laboratories. Also the *Enterococcus* strain in the sample appears with small convex pink and oxidase negative colonies on CCA. By experience they should be excluded.
- The distribution of the results was quite wide with a tail to the left. The dispersion was large. Two high outliers were present that could be a result of counting errors, i.e. multiplication with 10.

### Sample C

- No *E. coli* but the coliform bacteria, *Cronobacter sakazakii*, was present. This strain appeared together with a strain of *Aeromonas hydrophila* with, for coliform bacteria, typical colonies at 37 °C, i.e. with metallic sheen on LES and pinkish on CCA.
- The distribution of the accepted results was also here wide and the dispersion large. Seven false negative results were present, together with a tail of other low results. There are no clear reason for the false negative results (compare p. 14).
- *A. hydrophila* was a false positive strain but could be removed after confirmation with oxidase test because it is oxidase positive. In 9 of 42 cases the results for suspected coliform bacteria and coliform bacteria were identical. Either have the laboratories in these cases not excluded *A. hydrophila* after confirmation, or it is not even included among the suspected coliform bacteria.

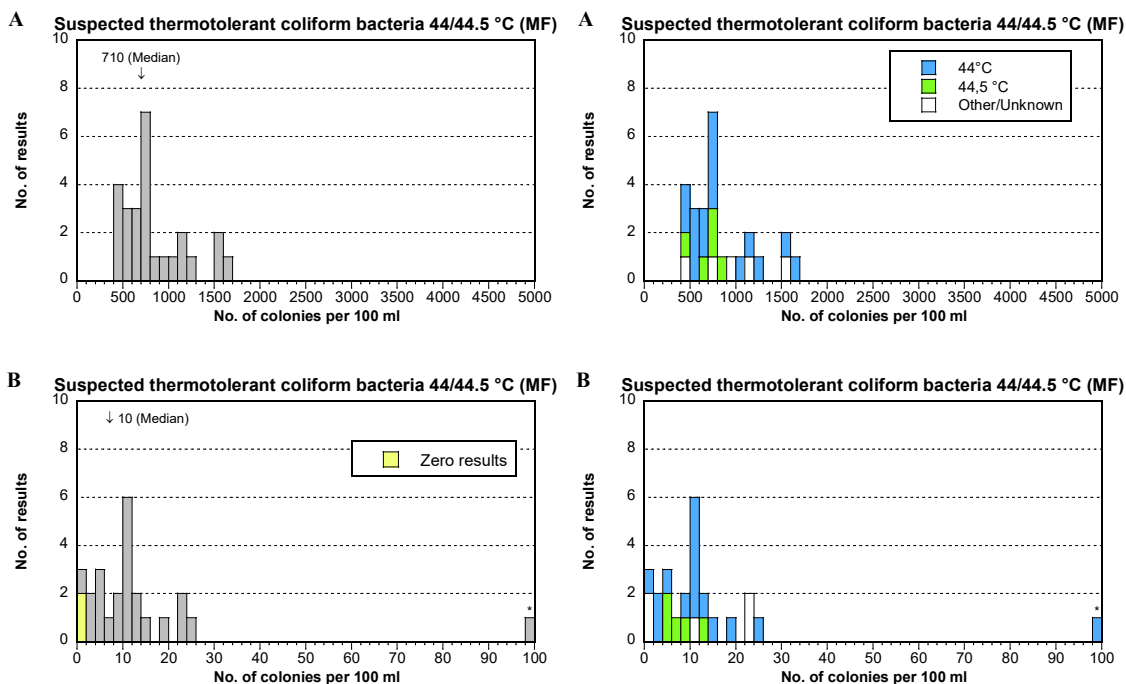
## Suspected thermotolerant coliform bacteria (MF)

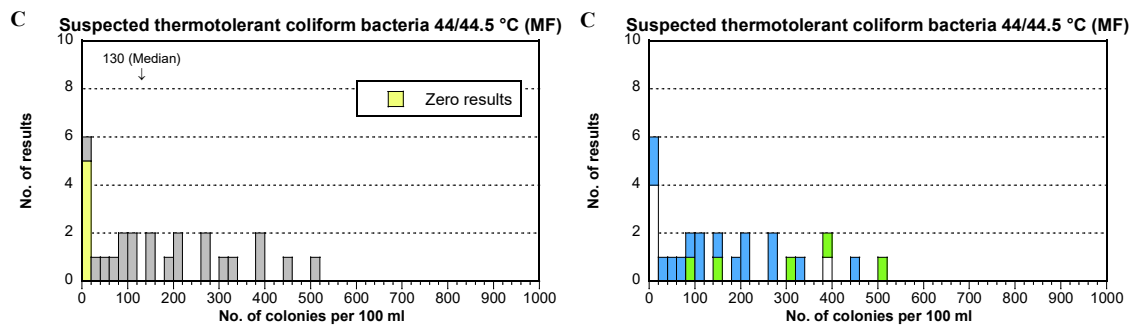
No evaluation in relation to performance is done for what is called suspected (not confirmed) colonies of a parameter. Therefore, no identification of outliers is done. The *medians* are then more robust than the means and are given in the table and in histograms. **Thus, the parameter is not included in the performance assessment.**

The only primary growth media used at 44 or 44.5 °C to identify suspected thermotolerant coliform bacteria is m-FC. In several cases within the group Other/Unknown primary media that are incubated at 36±2 °C have been used. In those cases 44 °C is used only for confirmation. This is not the intention with the parameter suspected thermotolerant coliform bacteria according to the definition in the instruction and on the website for the program. It is the typical colonies appearing on the membrane filter at 44/44.5 °C that should be reported. For the group Other/Unknown in sample B and C, where there are relatively low results, only minor fractions seem to have been reckoned as suspected thermotolerant coliform bacteria.

Incubation temp.	N	A					B					C					
		n	Med	CV	F	<	>	n	Med	CV	F	<	>	n	Med	CV	F
<b>Total</b>	26	26	792	-	-	-	25	11	-	-	-	26	117	-	-	-	
44 °C	16	16	799	-	-	-	15	15	-	-	-	16	127	-	-	-	
44,5 °C	5	5	668	-	-	-	5	7	-	-	-	5	265	-	-	-	
Other/Unknown	5	5	904	-	-	-	5	6	-	-	-	5	15	-	-	-	

Med = Median; used here instead of mean value because it describes "suspected" colonies





### Sample A

- Two coliform bacteria were included in the sample, of which the *E. coli* strain appears as a typical suspected thermotolerant coliform bacterium; that is with blue colonies at 44/44.5 °C on m-FC.
- The distribution of the 26 results was fairly good.

### Sample B

- Two coliform bacteria were included in the mixture, of which the *E. coli* strain appears as a typical suspected thermotolerant coliform bacterium; that is with blue colonies on m-FC at 44/44.5 °C. The strain of *H. alvei* doesn't grow on agar media at 44 °C.
- The distribution of the 25 results was fairly good but with some over-representation of low results. One very high result could be seen as an outlier but, no such evaluation is done.

### Sample C

- One strain of *C. sakazakii* together with a strain of *A. hydrophila* appear on media for coliform bacteria at 35-37 °C. The strain of *A. hydrophila* does not grow at 44 °C while the strain of *C. sakazakii* appear there with mainly blue-grey colonies on m-FC.
- The five zero results indicate that those laboratories didn't see the colonies as bluish and therefore didn't count them as thermotolerant coliform bacteria. Even without these zero results, there is some displacement to the left compared with the results for the coliform bacteria, where there is the same strain seen. Probably this is a result of a partial inhibition on m-FC due to the high temperature. This is quite normal for most bacteria strains growing on that medium at high temperature, even *E. coli*.

## *Escherichia coli* (MF)

To identify and quantify *E. coli*, confirmation is required when colonies are isolated from the primary cultivation media LES or m-FC. Depending on the method, tests for indole production and/or  $\beta$ -glucuronidase activity from oxidase negative presumptive strains are usually performed. A violet to blue colony on CCA indicates positive  $\beta$ -glucuronidase activity and is reckoned as a confirmed *E. coli*.

Corresponding reactions occur on other chromogenic media based on  $\beta$ -glucuronidase activity.

The primary growth media CCA, LES and others are used at  $36\pm 2$  °C and m-FC at  $44/44.5$  °C. In addition to incubation temperature, the results are also grouped by standard. For ISO 9308-1:2014 the incubation is at  $36\pm 2$  °C on CCA. For the standards from the Nordic countries (NS, SS and SFS) the majority of the results are from  $36\pm 2$  °C on LES but some are also from  $44/44.5$  °C on m-FC. Actually, only two Finnish laboratory have stated the standard SFS 4088 (m-FC) instead of SFS 3016 for the analysis of *E. coli*. One of these has used  $44$  °C and the other  $44.5$  °C

When all results are compared, there is no differences between the different incubation temperatures for any sample. For the standards there is an indication of a lower average for CCA compared to other groups in sample A. This time there is no difference in the dispersion (CV) between CCA and LES. In contrast, the results from the Finnish standard are for some reason showing a larger dispersion than those from other standards. It might be caused by the use of different confirmation principles and that a number of laboratories have confirmed at  $44.5$  °C, while the majority used  $44$  °C.

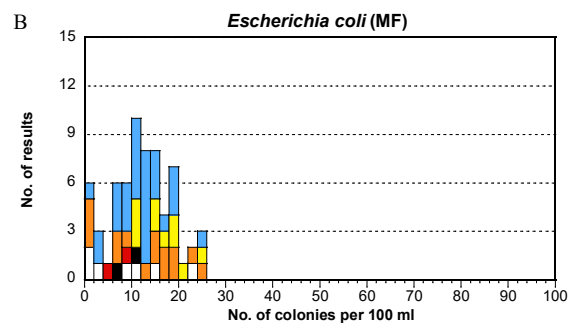
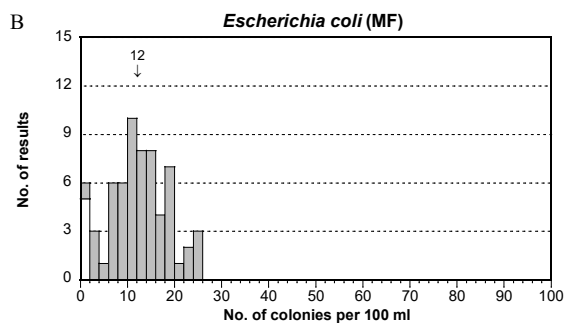
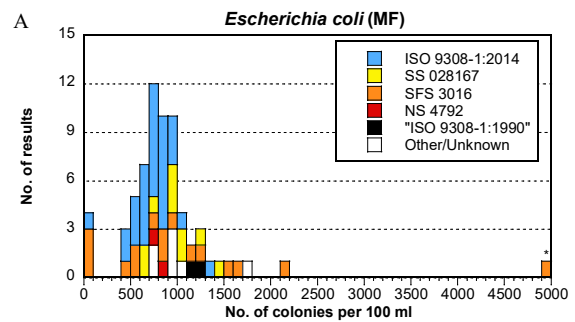
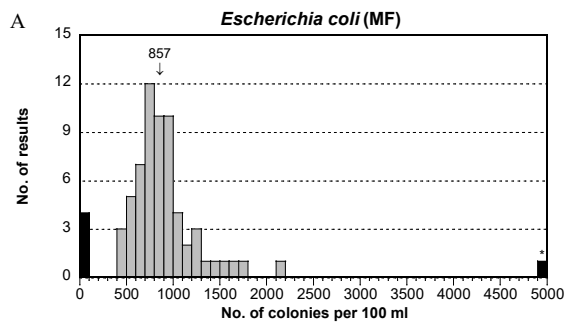
#### All results

Origin & Standard	N	A					B					C							
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >			
<b>Total</b>	<b>67</b>	<b>62</b>	<b>857</b>	<b>17</b>	<b>0</b>	<b>4</b>	<b>1</b>	<b>60</b>	<b>12</b>	<b>25</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>66</b>	<b>0</b>	<b>-</b>	<b>1</b>	<b>-</b>	<b>-</b>
<i>Colony origin</i>																			
$36 \pm 2$ °C	47	44	<b>853</b>	17	0	2	1	42	<b>12</b>	26	4	0	0	46	<b>0</b>	-	1	-	-
$44/44.5$ °C	9	8	<b>837</b>	16	0	1	0	7	<b>12</b>	25	1	0	0	9	<b>0</b>	-	0	-	-
$36 \pm 2$ & $44/44.5$ °C	10	9	<b>882</b>	19	0	1	0	10	<b>12</b>	27	0	0	0	10	<b>0</b>	-	0	-	-
Other/Unknown	1	1	-	-	0	0	0	1	-	-	0	0	0	1	<b>0</b>	-	0	-	-
<i>Standard</i>																			
ISO 9308-1:2014	30	29	<b>747</b>	12	0	1	0	28	<b>11</b>	23	1	0	0	30	<b>0</b>	-	0	-	-
SS 028167	10	10	<b>932</b>	13	0	0	0	10	<b>15</b>	16	0	0	0	10	<b>0</b>	-	0	-	-
SFS 3016 (4088)	16	12	<b>976</b>	<b>25</b>	0	3	1	13	<b>13</b>	<b>30</b>	2	0	0	15	<b>0</b>	-	1	-	-
NS 4792	2	2	-	-	0	0	0	2	-	-	0	0	0	2	<b>0</b>	-	0	-	-
"ISO 9308-1:1990"	2	2	-	-	0	0	0	2	-	-	0	0	0	2	<b>0</b>	-	0	-	-
Other/Unknown	7	7	<b>966</b>	16	0	0	0	5	<b>11</b>	33	2	0	0	7	<b>0</b>	-	0	-	-

#### Results from the analysis of "coliform bacteria" MF at $36\pm 2$ °C

Medium	N	A					B					C							
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >			
<b>Total</b>	<b>50<sup>#</sup></b>	<b>47</b>	<b>844</b>	<b>17</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>45</b>	<b>12</b>	<b>26</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>49</b>	<b>0</b>	<b>-</b>	<b>1</b>	<b>-</b>	<b>-</b>
m-Endo Agar LES	18	16	<b>1051</b>	18	0	1	1	17	<b>14</b>	26	1	0	0	17	<b>0</b>	-	1	-	-
Chromocult C Agar	29	28	<b>749</b>	12	0	1	0	27	<b>11</b>	25	1	0	0	29	<b>0</b>	-	0	-	-
Other/Unknown	3	3	-	-	0	0	0	1	-	-	2	0	0	3	<b>0</b>	-	0	-	-

# Compare table above – three more laboratories performed the analysis of *E. coli* than of coliform bacteria



### Sample A

- One strain of *E. coli* was present together with another thermotolerant coliform bacterium, *E. cloacae*. The colonies are typical for *E. coli* on LES and m-FC that are based on lactose fermentation. On CCA the colonies are typical blue, meaning that confirmation is not necessary and therefore normally not performed. Sometimes small colonies of *E. cloacae* may appear together with *E. coli* on m-FC at 44 °C. Confirmation is necessary for colonies from LES and m-FC.
- The distribution of the results was good and the dispersion small (CV; see p. 29) except the deviating results. Four low and one high outlier were present.
- For three of the low outliers there might be a miscalculation from the plate counted to the volume 100 ml. Alternatively, the confirmation may have failed.

### Sample B

- A typical strain of *E. coli* was included together with another atypical coliform bacterium, *H. alvei*. *H. alvei* shouldn't grow in broth at 44 °C, and is indole negative and lacking the enzyme  $\beta$ -glucuronidase. Thus, it cannot be mistaken for *E. coli*.
- The distribution of the results was good except a "tail" of low results, out of which five were false negative. Due to the low results, the dispersion (CV) was medium.

### Sample C

- No *E. coli* was included but another coliform bacterium, *C. sakazakii*, was present together with the coliform-like bacterium, *A. hydrophila*. The latter is oxidase positive. *C. sakazakii* is indole negative and has no activity of  $\beta$ -glucuronidase. Thus, neither stain can be mistaken for *E. coli* after confirmation.
- One false positive result was reported.

## Coliform bacteria & *E. coli* (rapid methods, MPN)

The rapid method used for both these parameters was exclusively Colilert® Quanti-Tray® from the manufacturer IDEXX Inc. with incubation at 35, 36 or 37 °C. Out of the about 60 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 wells (a few of which, probably incorrectly, have reported 96 wells). The laboratories often analysed both diluted and undiluted samples. Yellow wells (ONPG positive;  $\beta$ -galactosidase activity shown) will be interpreted as coliform bacteria and yellow wells also exhibiting fluorescence (MUG positive;  $\beta$ -glucuronidase activity shown) will be interpreted as *E. coli*.

When comparing trays with different number of wells (see the table) as well as different incubation temperatures it is clear that the differences are small and inconsistent. Differences based on stated maximum incubation time were also small.

In this round it is clear that the two laboratories using "Wrong method" ("most probable numbers" in connection with a multiple tube method) instead of a rapid kit method obtained some low or high deviating results. Further, even most averages for the accepted results are low according to the table. The third laboratory in the group "Wrong method" used a presence/absence method with an evaluable result only for *E. coli* in sample C, where no deviating results were seen.

### Coliform bacteria, Rapid method with MPN

Principle	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, Rapid meth.</b>	62	61	1805	11	0	0	0	60	21	26	0	0	1	60	350	16	0	1	0
Colilert-18, 51 wells	12	11	1804	12	0	0	0	12	19	21	0	0	0	11	313	23	0	0	0
Colilert-18, 97 wells	45	45	1773	11	0	0	0	43	22	27	0	0	1	44	358	13	0	1	0
Colilert-18, 51 & 97	2	2*	2177	8	0	0	0	2*	25	15	0	0	0	2*	389	4	0	0	0
Colilert-24, ? wells	3	3*	2072	7	0	0	0	3*	14	29	0	0	0	3*	348	33	0	0	0
<b>Wrong method<sup>#</sup></b>	2	1*	920	-	0	0	1	2*	10	77	0	0	0	1*	70	-	1	0	0

### *E. coli*, Rapid method with MPN

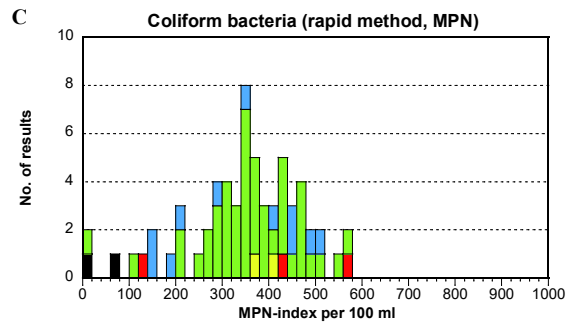
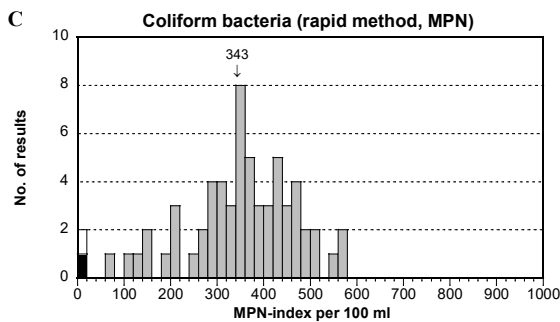
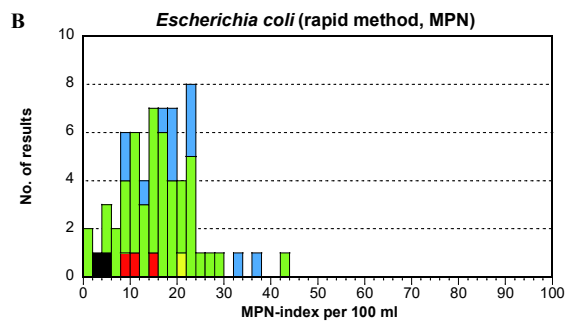
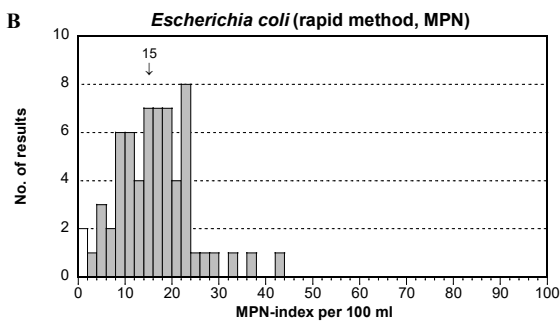
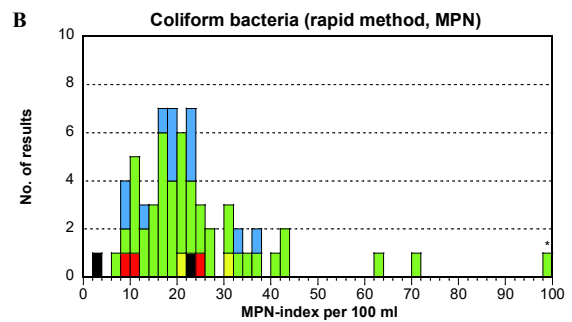
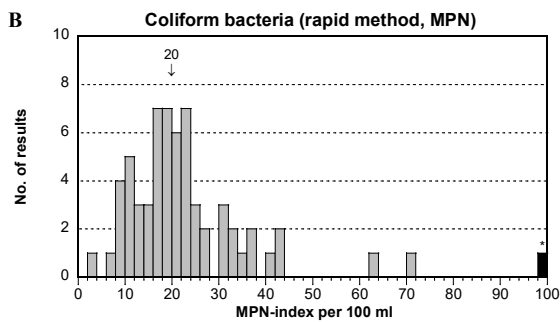
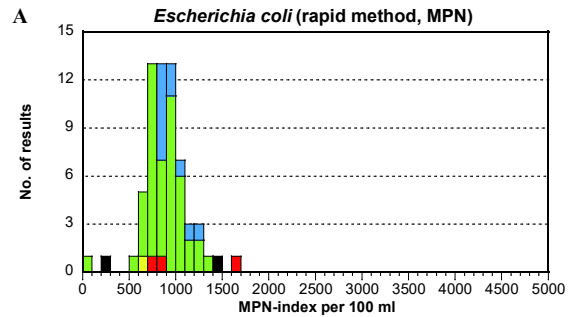
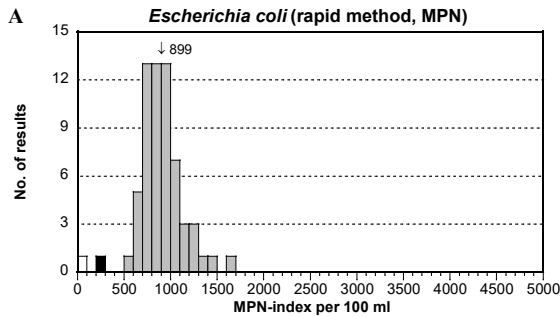
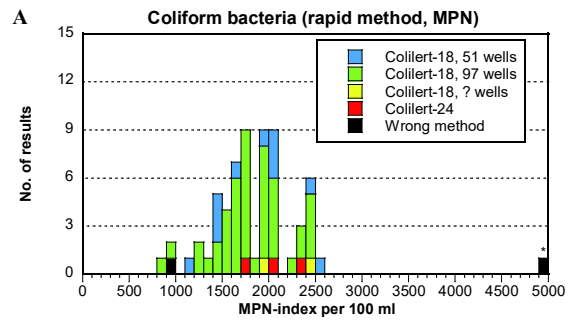
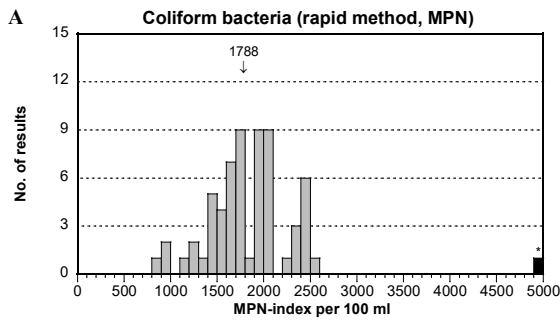
Principle	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, Rapid meth.</b>	61	60	14	13	1	0	0	61	0	-	0	-	-	59	41	10	0	1	1
Colilert-18, 51 wells	12	11	939	7	0	0	0	12	19	22	0	0	0	12	0	-	0	-	-
Colilert-18, 97 wells	46	45	876	10	1	0	0	43	15	22	2	0	0	45	0	-	1	-	-
Colilert-18, 51 & 97	1	1*	687	-	0	0	0	1*	20	-	0	0	0	1	0	-	0	-	-
Colilert-24, ? wells	3	3*	1026	23	0	0	0	3*	11	14	0	0	0	3	0	-	0	-	-
<b>Wrong method<sup>#</sup></b>	3	1*	1400	-	0	1	0	2*	3	32	0	0	0	3	0	-	0	-	-

\* Mean value is given for comparison despite few results

# In two cases no rapid kit method but a multiple tube method based on lactose fermentation, in the third case a qualitative presence/absence method

### Sample A

- The strains of *E. coli* and *E. cloacae* grow in the medium and have the enzyme  $\beta$ -galactosidase. Therefore, they are detected as coliform bacteria by methods based



on this enzyme (ONPG positive) e.g. Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup> where ONPG is a substrate.

- The strain of *E. coli* has the enzyme  $\beta$ -glucuronidase and is detected as *E. coli*.
- The distributions of the results were good and the dispersions small (CV; see p. 29). The only deviating results were a high outlier for coliform bacteria, as well as a low outlier and one false negative result for *E. coli*.
- The averages for both coliform bacteria and *E. coli* were only somewhat higher than for the corresponding analyses with the MF technique (compare p. 6 and 10).

### Sample B

- The strains of *E. coli* and *H. alvei* grow in the medium and possess the enzyme  $\beta$ -galactosidase. Therefore, they are detected as coliform bacteria by methods based on this enzyme (ONPG positive) e.g. Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup> where ONPG is a substrate. *E. coli* has the enzyme  $\beta$ -glucuronidase and is detected as *E. coli*.
- The activity of  $\beta$ -galactosidase is weak in *H. alvei*, often resulting in negative outcome after 18 hours and positive only after 22 hours. This species is completely lacking the enzyme  $\beta$ -glucuronidase and, is thus not detected as *E. coli*.
- The distribution of the results for coliform bacteria is not as dispersed as for the MF-method (lesser CV) but the means are still the same. You could expect a somewhat higher mean with the rapid method as at least the colonies of *H. alvei* on LES with the MF method are not reckoned as coliform bacteria. The outcome is instead indicating that quite a number of laboratories also with the rapid method have not detected *H. alvei* as positive. You might then question if the final reading has been earlier than after 22 hours.
- One high outlier was present for coliform bacteria.
- The average for *E. coli* was somewhat higher with the rapid method compared to the MF method, which is often the case. The dispersions were similar and medium in both cases. Two false negative results were present.

### Sample C

- In this sample only one coliform bacterium, *C. sakazakii*, was present. It has the enzyme  $\beta$ -galactosidase and is detected as a coliform bacterium. *A. hydrophila* that was included in the sample, and could be taken for a coliform bacterium by the MF method before confirmation, is not detected as such by Colilert<sup>®</sup>.
- *C. sakazakii* is lacking the enzyme  $\beta$ -glucuronidase and is not detected as *E. coli*.
- The distribution of the results was good with small dispersion in average. One low outlier and one false negative result were the only deviating results.
- The average for the accepted results of the coliform bacteria was about 50 % higher with the rapid method compared to the MF method (see p. 7). This together with the tail of low results and false negative values by the MF method indicates that some laboratories had difficulties in interpreting the colonies of *C. sakazakii* by that method. Some of the laboratories seem to have been interpreted them as not being coliform bacteria.





## Intestinal enterococci (MF/MPN)

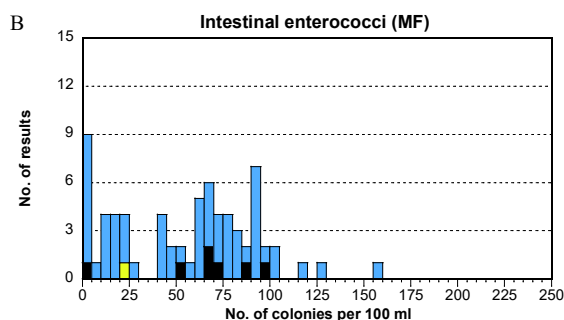
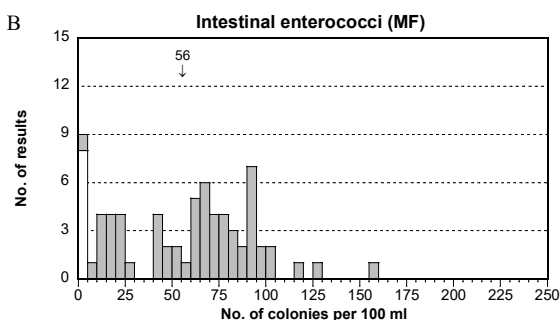
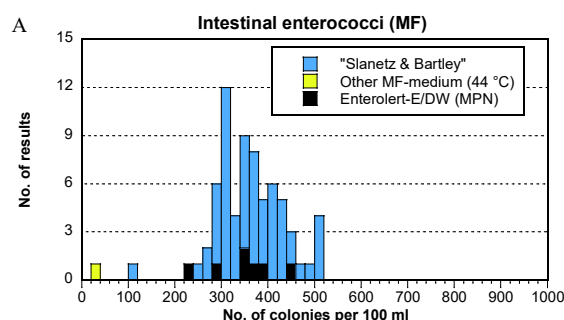
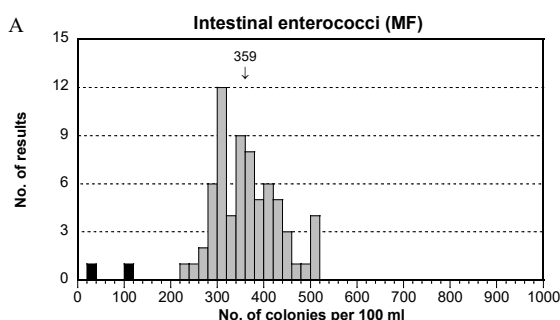
The MF method used for intestinal enterococci is almost exclusively EN ISO 7899-2:2000. In seven cases methods with another reference, like "according to manufacturer's instruction", have been stated. The primary growth medium was m-Enterococcus Agar (Slanetz & Bartley), here designated m-Ent, except in these seven cases and one more. In this last case the laboratory used Rapid Enterococcus Agar at 44 °C without confirmation. In the other seven cases the rapid method Enterolert<sup>®</sup>-E (Idexx Inc.) was used by five and Enterolert<sup>®</sup>-DW (Idexx Inc.) by two laboratories. The incubation temperature was 41 °C in six of these laboratories but 41.5 °C in the seventh. In all cases with the MF method and m-Ent the incubation temperature was 35, 36 or 37 °C.

The prominent method difference is the MF-method versus the rapid method. No general trend can be seen, instead the differences are sample specific (see below). There are some variants of the confirmation step for the MF methods. However, no general differences in the results relating to that could be seen. In sample B the dispersion is large for the MF methods irrespective of the variants (not shown).

Method/Medium	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	<b>70</b>	<b>68</b>	<b>359</b>	<b>9</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>62</b>	<b>56</b>	<b>31</b>	<b>8</b>	<b>0</b>	<b>0</b>	<b>66</b>	<b>0</b>	<b>-</b>	<b>4</b>	<b>-</b>	<b>-</b>
<i>EN ISO 7899</i>	63	61	361	9	0	2	0	56	55	33	7	0	0	59	0	-	4	-	-
Slanetz & Bartley	62	61	361	9	0	1	0	55	55	33	7	0	0	58	0	-	4	-	-
Other/Unknown	1	0	-	-	0	1	0	1*	22	-	0	0	0	1	0	-	0	-	-
<i>Rapid method<sup>#</sup>, MPN</i>	7	7	345	10	0	0	0	6	74	10	1	0	0	7	0	-	0	-	-

\* Mean value is given for comparison despite only one result

# Two variants of Enterolert<sup>®</sup> – no confirmation was performed



### Sample A

- A strain of *Enterococcus faecalis* was present. The distribution of the results was good with very small dispersion (CV; see page 29). The colonies are brown-red on m-Ent and are normally confirmed as enterococci without problem.
- The results by Enterolert<sup>®</sup> were not deviant from those by the MF-method.
- Two low outliers were present, one of which was by the Rapid Enterococcus Agar (44 °C).

### Sample B

- A strain of *Enterococcus faecium* was included. The distribution of the results showed two peaks (with 23 and 47 results, respectively) and was therefore wide with large dispersion. The colonies were brown-red in varying degree on m-Ent, the darkest colonies were also often even the biggest. Eight false negative results were present.
- In many instances, the darkest colonies were the only ones giving clear positive confirmation on Bile Esculin Azide Agar (BEAA). Thus, many colonies were taken as negative in the confirmation. This is the probable cause to the varying results. Extended confirmation time didn't necessarily give more positive colonies.
- The median is higher for suspected intestinal enterococci (81 cfu/100 ml) compared to for all confirmed results (66 cfu/100 ml). The median for the rightmost peak alone of the confirmed results is 74 cfu/100 ml. ***All results, except the false negative ones, are considered as acceptable due to the variation in colony colour and confirmation outcome.***
- The results by Enterolert<sup>®</sup> were generally higher (74 cfu/100 ml) because they were absent in the peak with the 23 lowest values, except the false negative one. This false negative results was obtained by the only laboratory that incubated at 41.5 °C. In principle all bacteria were detected as intestinal enterococci by Enterolert<sup>®</sup>, corresponding to the suspected ones mentioned above.

### Sample C

- No enterococcus strain was included but the strain of *Staphylococcus saprophyticus* may sometimes appear on m-Ent with small, often brownish, colonies after 2 days.
- Four false positive results were present despite the small atypical colonies. No blackening at all was seen on BEAA during confirmation (see annex C).

## *Pseudomonas aeruginosa* (MF/MPN)

EN ISO 16266:2008 with or without modification was used by 46 of the 59 laboratories that reported results. One laboratory stated the identical, but since long time withdrawn, CEN standard EN 12780:2002 without modification. Pseudalert<sup>®</sup> (Idexx Inc.) was reported by 13 laboratories. The incubation was in eleven of these cases done at 38 °C and in 2 cases at 37 °C. For the MF methods the incubation was done at 35, 36 or 37 °C.

Since unhealthy substances like mercury are included, many laboratories have replaced the confirmation tests in the standard by another method. The major modification of the method therefore concerns the confirmation. When only typical yellow-green to blue-green colonies are present, no confirmation needs to be done. In those cases there is no principal difference between what is counted whether "mod." is stated for the method or not.

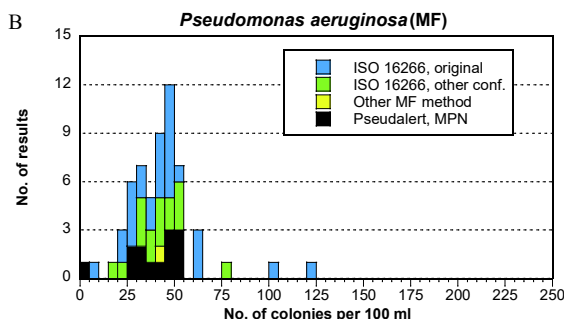
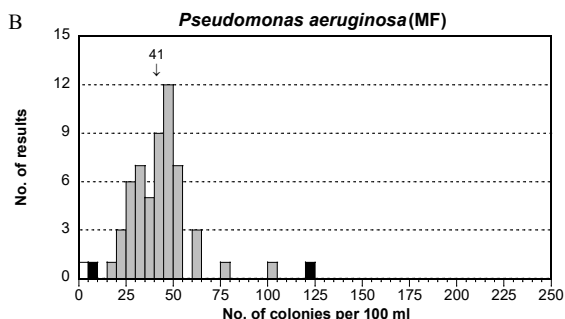
The colonies in sample C were typical, meaning no confirmation was necessary. Those in sample B were a bit more yellow-green, and could normally be deemed as *Pseudomonas aeruginosa* without confirmation. The colonies were clearly fluorescing in UV light in both the samples.

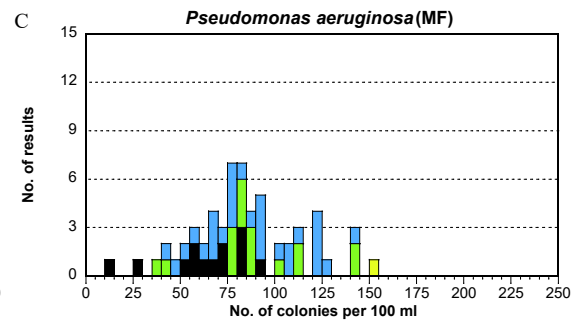
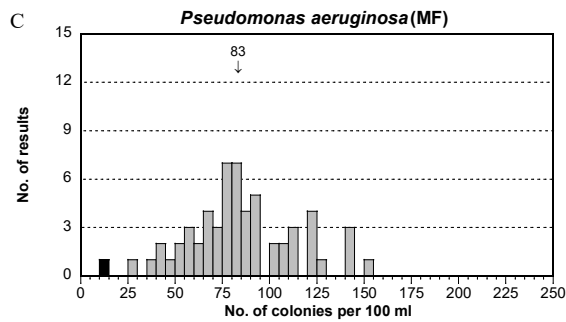
In sample C the average was lower by Pseudalert<sup>®</sup> than by the MF-methods, but the dispersions (CV) were approximately the same. In sample B no difference between methods could be seen.

Standard/Method	N	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
<b>Total</b>	<b>59</b>	<b>54</b>	<b>0</b>	<b>-</b>	<b>4</b>	<b>-</b>	<b>55</b>	<b>41</b>	<b>16</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>58</b>	<b>83</b>	<b>17</b>	<b>0</b>	<b>1</b>	<b>0</b>
Membrane filtration	46	41	0	-	4	-	43	41	18	0	1	1	46	88	16	0	0	0
ISO 16266 <sup>a</sup>	29	24	0	-	4	-	26	42	18	0	1	1	29	87	15	0	0	0
ISO 16266, mod. <sup>b</sup>	16	16	0	-	0	-	16	41	18	0	0	0	16	86	17	0	0	0
Other	1	1	0	-	0	-	1	-	-	0	0	0	1	-	-	0	0	0
Pseudalert <sup>®</sup> , MPN	13	13	0	-	0	0	12	41	12	1	0	0	12	66	14	0	1	0

a Modification not stated for confirmation; includes EN 12780:2002

b Alternative confirmation performed, e.g. Maldi-TOF, API, phenanthroline test





### Sample A

- There was no *P. aeruginosa* in the sample but instead pale yellow colonies of *Burkholderia cepacia*. Four laboratories reported them as suspected *P. aeruginosa*. Of these, one also reported the result as false positive for *P. aeruginosa*. For the remaining laboratories, confirmation has likely been done with a correct negative outcome.
- Four false positive results were reported.

### Sample B

- One strain of *P. aeruginosa* with relatively light yellow-green colonies on PACN was included. The colonies showed a clear fluorescence under UV light. Due to the green colour, no confirmation is necessary according to the standard.
- The results were well accumulated and the distribution therefore good with a small dispersion (CV; see page. 29).
- On false negative result as well as one low and one high outlier were present.

### Sample C

- One strain of *P. aeruginosa* with typical blue-green colonies on PACN was included. The colonies showed a clear fluorescence under UV light. No confirmation was necessary according to the standard due to the colour.
- The results appear more dispersed than they are due to the scale of the x-axis. The distribution was good, which can be seen from the small dispersion.
- One low outlier was present.

## Culturable microorganisms 22 °C, 3 days

Eighty-three of the 85 laboratories performing the analysis reported EN ISO 6222:1999 as method, which prescribes the use of Yeast extract Agar (YeA). Eight laboratories used Plate Count Agar instead, simultaneously stating the use of EN ISO 6222:1999. One laboratory used YeA and yet another "Standard Methods Agar" (= PCA) in conjunction with Standard methods [5]. These two comprise the group "Other method". The majority of the laboratories have claimed counting both bacterial and fungal colonies. Only four state that they don't count fungi and three others that they count yeasts but not moulds.

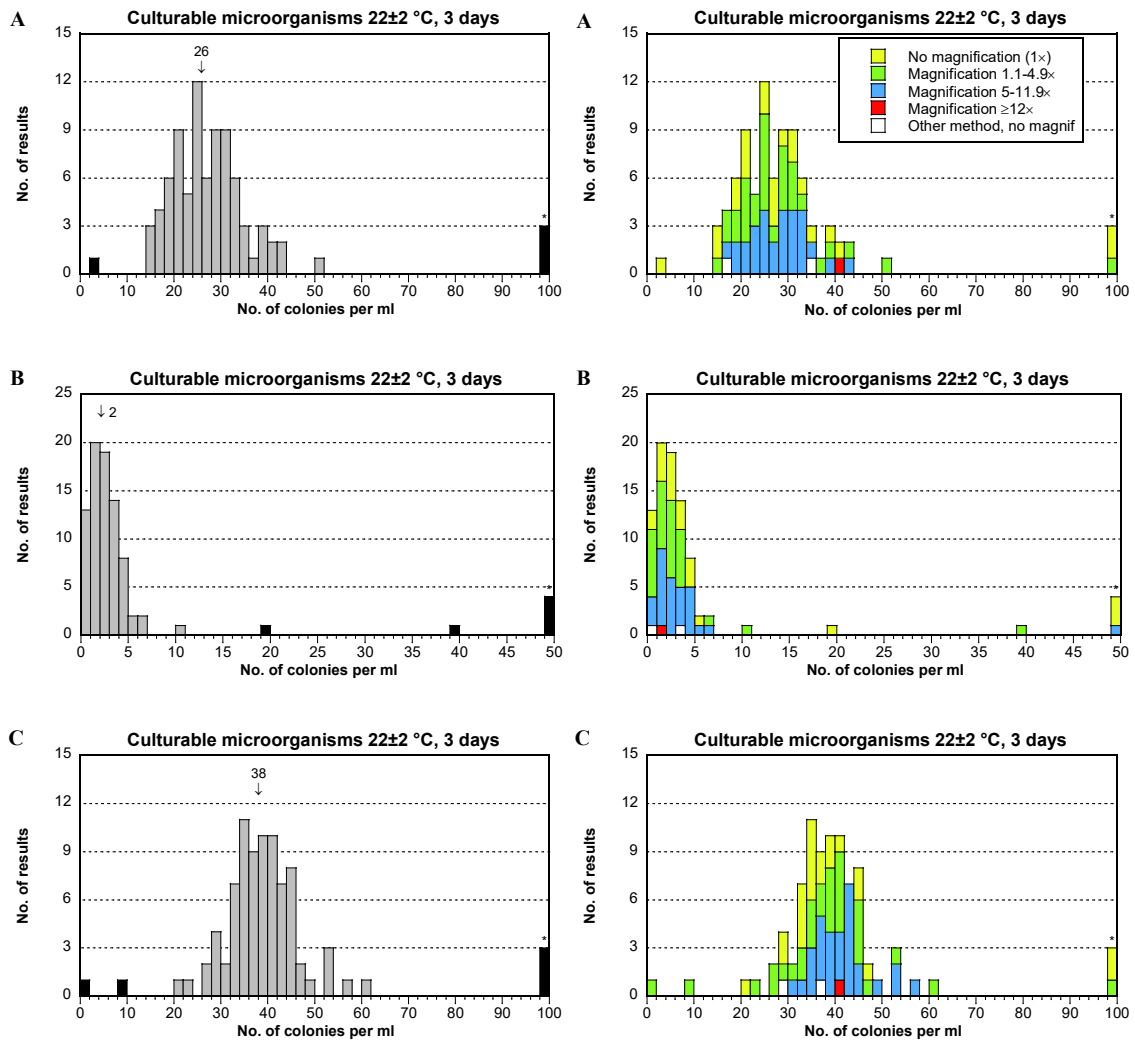
Since all except two laboratories refer to EN ISO 6222:1999, differences among method variants are relevant to discuss only for these. Results are shown for culture media and magnification at reading.

It is difficult to find any consistent method difference. Both in sample A and B, Plate Count Agar gave larger dispersion (CV) than YeA, probably due to the few results for PCA. No general difference was seen in relation to magnification. There might be a tendency to higher results in the two groups with the highest magnification, but it is not certain. The culturable microorganisms at 22 °C were easy to count in all samples. There were no small colonies present that could be difficult to discern. This explains why there were only minute differences when various magnifications were used for counting.

The distributions of the results were good for all samples and the dispersions were small (CV; see p. 29) in sample A and C. In sample B, however, the relative dispersion was very large due to the very low average content. This is quite normal. Some deviating results were reported for each sample.

Group of results	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, all results</b>	<b>85</b>	<b>81</b>	<b>26</b>	14	0	1	3	<b>79</b>	<b>2</b>	56	0	0	6	<b>80</b>	<b>38</b>	9	0	2	3
<b>EN ISO 6222</b>	<b>83</b>	<b>79</b>	<b>26</b>	13	0	1	3	<b>77</b>	<b>2</b>	54	0	0	6	<b>78</b>	<b>38</b>	9	0	2	3
<u>Medium</u>																			
Yeast extract Agar	75	72	<b>26</b>	13	0	1	2	69	<b>2</b>	53	0	0	6	73	<b>38</b>	9	0	1	1
Plate Count Agar	8	7	<b>28</b>	19	0	0	1	8	<b>1</b>	69	0	0	0	5	<b>37</b>	8	0	1	2
<u>Magnification</u>																			
None	22	19	<b>25</b>	15	0	1	2	18	<b>2</b>	46	0	0	4	20	<b>35</b>	8	0	0	2
1.1–4.9×	31	30	<b>26</b>	15	0	0	1	30	<b>1</b>	68	0	0	1	28	<b>38</b>	11	0	2	1
5–11.9×	29	29	<b>27</b>	11	0	0	0	28	<b>2</b>	47	0	0	1	29	<b>41</b>	7	0	0	0
> 12×	1	1*	<b>40</b>	–	0	0	0	1*	<b>1</b>	–	0	0	0	1*	<b>40</b>	–	0	0	0
<b>Other method</b>	<b>2</b>	<b>2*</b>	<b>25</b>	–	0	0	0	<b>2*</b>	<b>1</b>	–	0	0	0	<b>2*</b>	<b>32</b>	–	0	0	0

\* Mean value is given for comparison despite few results



### Sample A

- The colonies consist of all the four bacteria in the sample. The two coliform bacteria are in majority.
- The distribution of the results was good, with one low and three high outliers.

### Sample B

- The few colonies comprise all bacteria included, but mainly *E. faecium*.
- The distribution of the results was good despite the low average content. Six high outliers were present, out of which five were unreasonably high.

### Sample C

- The colonies mainly consist of *S. saprophyticus*, but the three other strains also contributed with a few colonies.
- The distribution of the results was good but with two low and three high outliers. Two of the three high outliers are unreasonably high.

## Culturable microorganisms 36 °C, 2 days

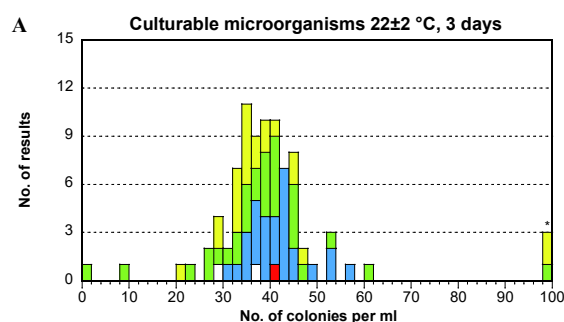
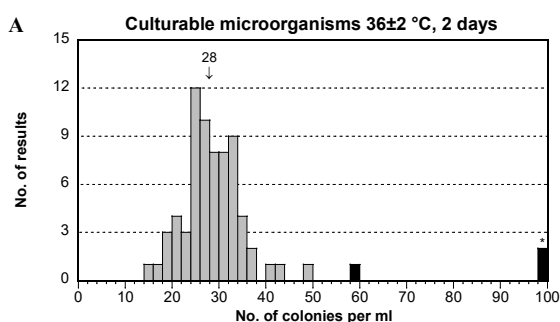
Sixty-nine of the 71 laboratories have stated the use of EN ISO 6222:1999. The two laboratories in the group "Other method" in the table have stated Standard Methods [5]. Five laboratories have reported Plate Count Agar (PCA), all in combination with EN ISO 6222:1999. The values for PCA together with EN ISO 6222:1999 are shown as comparison in the table, despite only 4 values in sample C.

As for the analysis at 22 °C, comparisons of method variants are relevant to discuss only when EN ISO 6222:1999 was used. Also here, the results are presented in relation to culture media and magnification for reading.

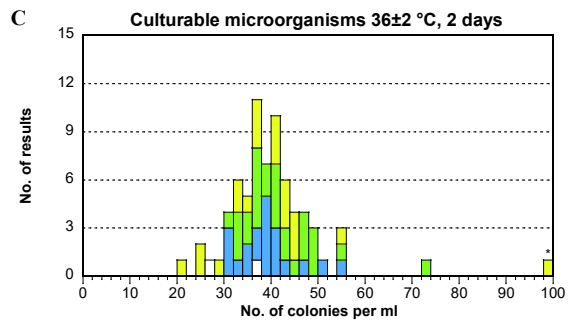
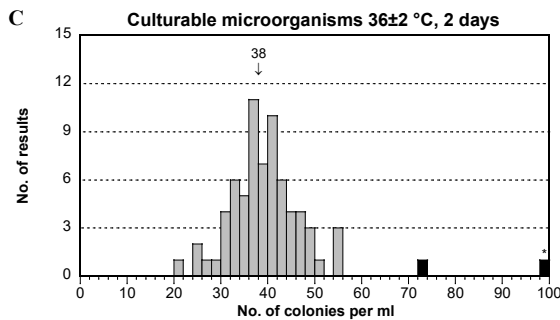
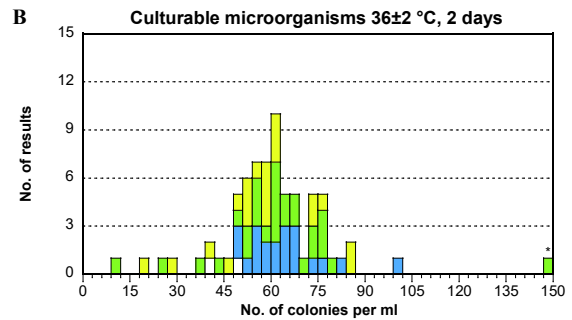
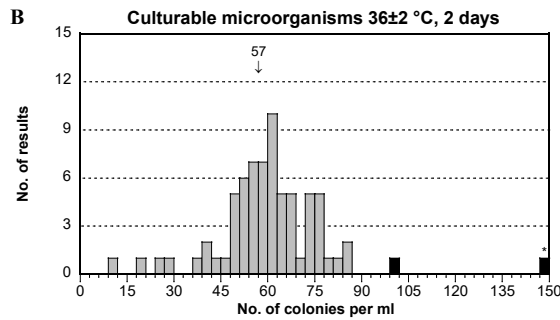
The five laboratories with PCA in sample B show a somewhat lower average result. However, this could be attributed to the way that was used for reading the plates. Three of the laboratories have stated reading without magnification. The average results for "Other method" are somewhat lower than for other groups for all samples.

Group of results	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, all results</b>	<b>71</b>	<b>68</b>	<b>28</b>	11	0	0	3	<b>69</b>	<b>57</b>	14	0	0	2	<b>69</b>	<b>38</b>	9	0	0	2
<i>EN ISO 6222</i>	69	66	28	11	0	0	3	67	58	14	0	0	2	67	39	9	0	0	2
<i>Medium</i>																			
Yeast extract Agar	64	62	28	11	0	0	2	62	59	14	0	0	2	63	39	9	0	0	1
Plate Count Agar	5	4*	25	7	0	0	1	5	49	10	0	0	0	4*	38	4	0	0	1
<i>Magnification</i>																			
None	21	20	28	14	0	0	1	21	56	16	0	0	0	20	37	11	0	0	1
1.1–4.9×	28	27	27	10	0	0	1	27	57	16	0	0	1	27	40	8	0	0	1
5–11.9×	20	19	29	8	0	0	1	19	62	7	0	0	1	20	38	8	0	0	0
> 12×	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Other method</i>	2	2*	24	–	0	0	0	2*	44	–	0	0	0	2*	31	–	0	0	0

\* Mean value is given for comparison despite few results







### Sample A

- All strains in the sample appeared as culturable microorganisms at 36±2 °C. No particular problems seemed to be present.
- The distribution of the results was good with a very small to small dispersion (CV; see page 29). Three high outliers were present.

### Sample B

- All strains in the sample will grow at 36±2 °C. The considerably higher average here compared to at 22 °C is caused by the strain of *S. capitis* that is present in highest concentration and which grows at 36 °C but not at 22 °C.
- The distribution shows, as in previous similar samples (latest September 2018), a small tail of low results. The reason for these is unclear. Possibly, some of the *S. capitis* colonies may not be considered as colonies under the magnification used.
- The lowest results were not objectively identified as deviating ones, although they could be reckoned as such. The relative dispersion of the accepted results was small despite these low results.
- Two high outliers were identified.

### Sample C

- All strains in the sample appeared as culturable microorganisms at 36±2 °C. No particular problems seemed to be present.
- The distribution of the results was very good with a very small dispersion.
- Two high outliers were present.

## **Outcome of the results and laboratory assessment**

### **General information about reported results**

The distributions of results for the respective analysis are shown in the histograms. A box plot (see below) gives a summarizing image of all the results of a laboratory, except false results. The number of false results and outliers are given below the plot for each laboratory. These values are highlighted with bold text on yellow background in annex A. The limit values for lowest and highest accepted results are given for each analysis in the summarizing lines at the end of annex A, together with the measurement uncertainty of the mean.

### **Base for assessment of the performance**

The laboratories are not grouped or ranked in relation to their performances. The performance of an individual laboratory can be broadly assessed by the numbers of false results and outliers.

*Generally, the laboratories that did not report their results in due time need to evaluate their results themselves. This can be done by comparison with the results of all other laboratories, by looking in tables, figures and annex A.*

### **Mixed up results and other practical errors**

Fourteen laboratories have more than one deviating result. When whole samples seem to have been mixed up, the corresponding sample numbers are crossed out in annex A. One laboratory (8663) seems to have mixed up the vials from sample B and C. In one case it seems that two results from a parameter have been mixed up. A number of laboratories seem to have performed individual incorrect calculations from their colony readings to the final concentrations.

### **Z-scores, box plots and deviating results for each laboratory**

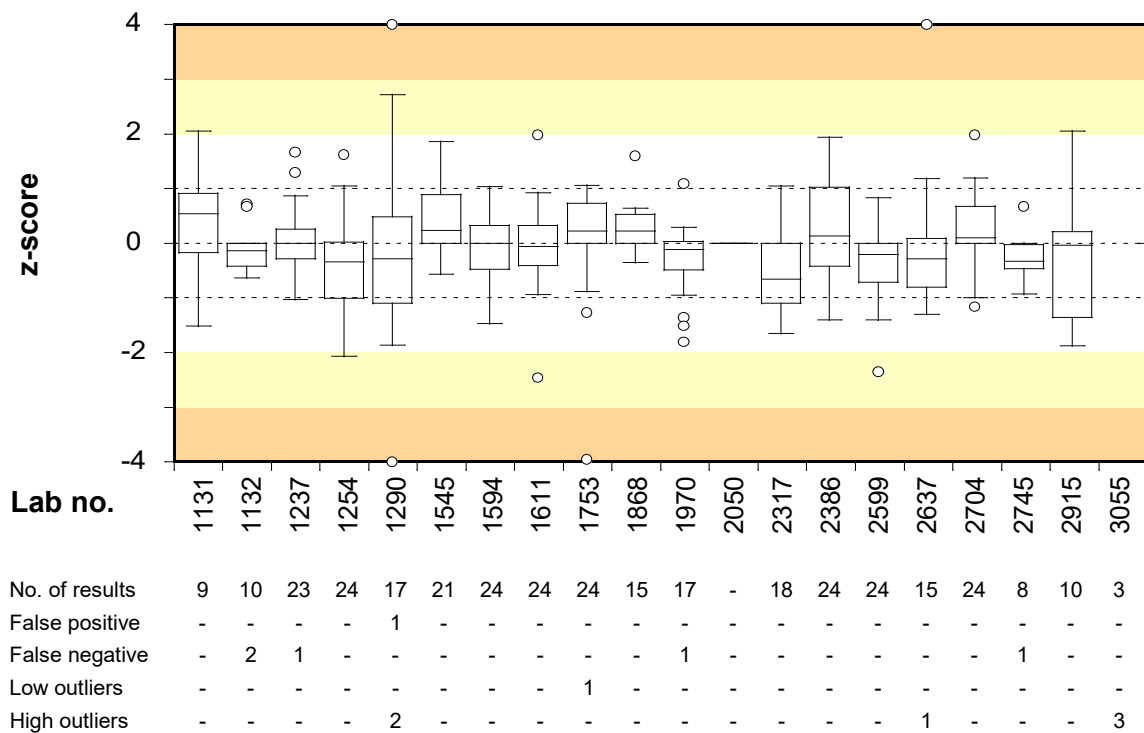
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are reported in annex B but are not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control charts etc. For interpretation and calculation of z-scores, see the explanation to annex A and the scheme protocol [1].

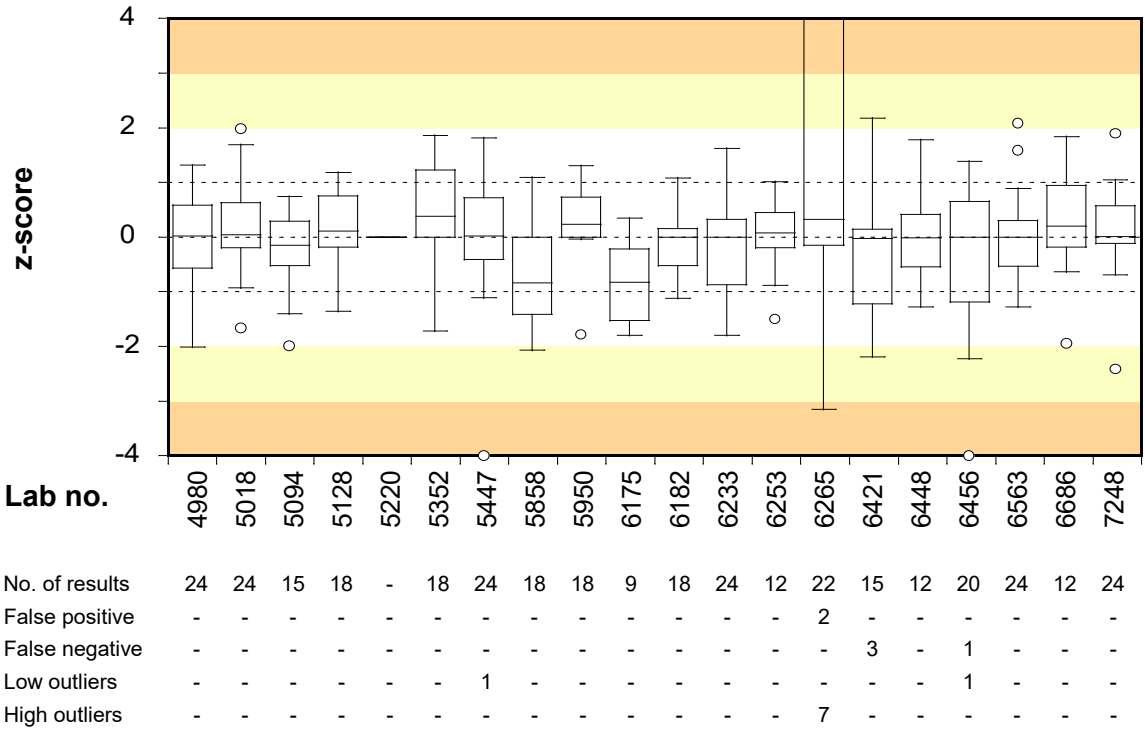
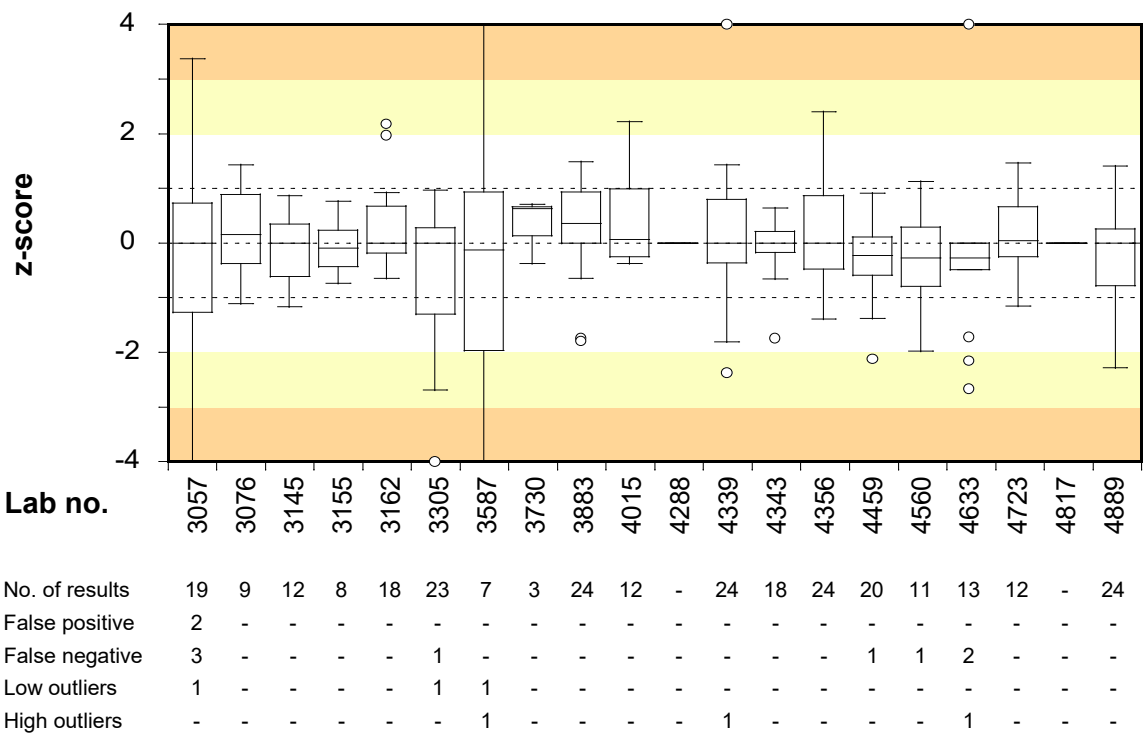
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better the agreement is between the laboratory's results and the means from all laboratories.

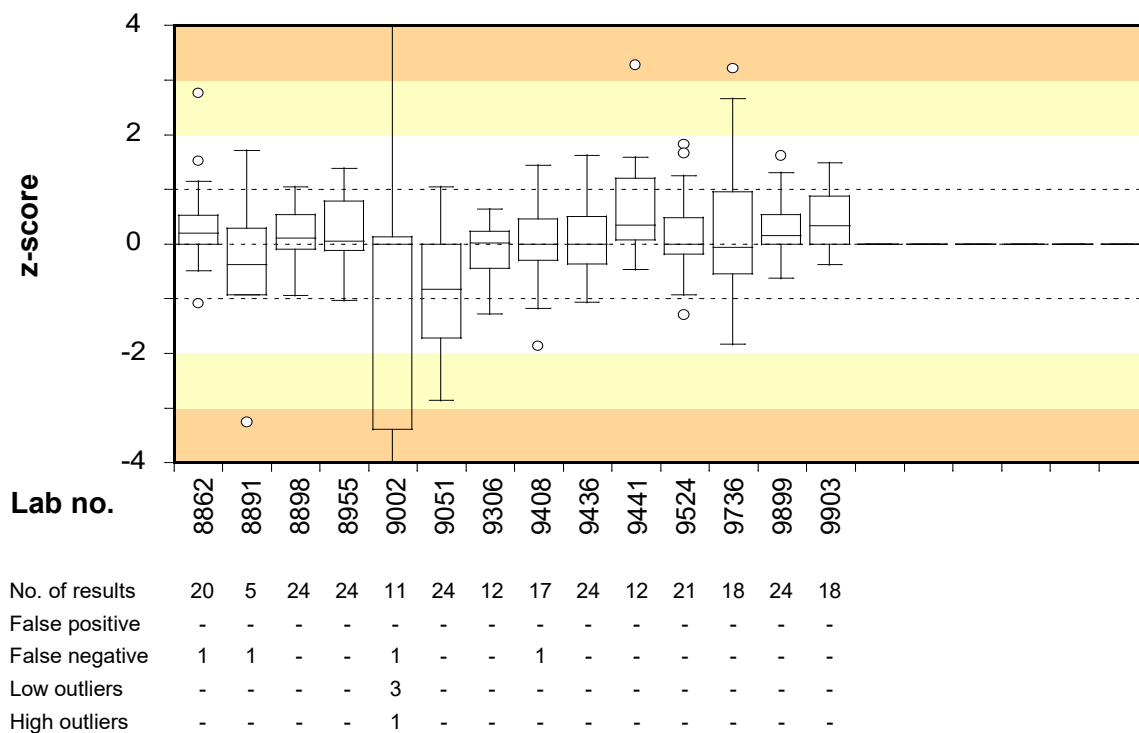
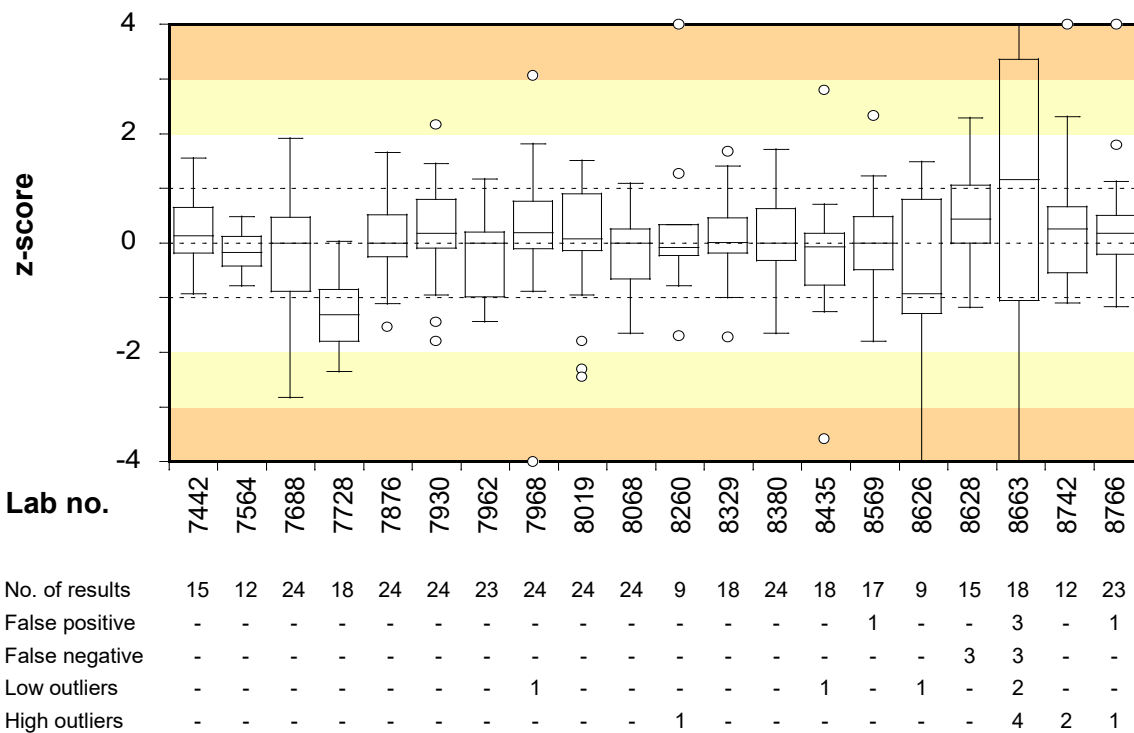
**Box plots and numbers of deviating results for each participating laboratory**

- z-scores are calculated from the formula  $z = (x - mv) / s$  (see annex A).
- A correct result "zero" will get  $z = 0$  when there is no target organism present.
- False results do not generate z-scores and are not included in 'No. of results'.
- The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as the rest of the results for each parameter.
- z-scores  $> +4$  and  $< -4$  have in the plots been set to  $+4$  and  $-4$ , respectively.
- The numbers of false positive and false negative results are given in the table under the plots together with the numbers of outliers.
- The horizontal line in each box indicates the median for the laboratory.
- The box includes 25 % of the results above and below the median. The lines protruding from the box and/or the circles embrace the remaining 50 % of the results, false results excluded.
- A circle is for technical reasons shown when a result is to a certain degree deviating\* from the rest. This alone does not mean it is an outlier.
- The background is divided into coloured fields to simplify localization of the laboratory results.

\*  $< [\text{smallest value of the box} - 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$  or  $> [\text{largest value of the box} + 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$







## Test material, quality controls and processing of data

### Description of the test material

This round comprised three test items with different microorganism compositions. The test material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd [2]. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition and approximate concentrations in the samples obtained at the Swedish Food Agency are listed in table 2. The participating laboratories were assigned to perform the analyses according to the methods routinely used by them.

The test material is primarily suited to the EN ISO methods for analyses of drinking water referred to in the European Drinking water directive [4] and its updates [6]. Alternative methods and other standards can usually be used without any problem.

**Table 2** *Microorganisms present in the samples*

Sample <sup>1</sup>	Microorganisms	Strain collection no.		cfu/100 ml <sup>2</sup>
		SLV (own)	Reference <sup>3</sup>	
A	<i>Escherichia coli</i>	165	CCUG 43600	1000
	<i>Enterobacter cloacae</i>	451	CCUG 30205	1000
	<i>Enterococcus faecalis</i>	051	CCUG 45101	300
	<i>Burkholderia cepacia</i>	042	–	700
B	<i>Escherichia coli</i>	082	CCUG 45097	1400
	<i>Hafnia alvei</i>	566	new strain	2800
	<i>Enterococcus faecium</i>	459	CCUG 35172	25
	<i>Pseudomonas aeruginosa</i>	455	CCUG 30209	3
	<i>Staphylococcus capitis</i>	463	CCUG 35173	8
C	<i>Cronobacter sakazakii</i>	419	–	50
	<i>Aeromonas hydrophila</i>	533	CCUG 48892	44
	<i>Pseudomonas aeruginosa</i>	395	CCUG 43596	52
	<i>Staphylococcus saprophyticus</i>	013	CCUG 45100	20*

1 The links between the samples and the randomised sample numbers are shown in annex A; the analyses were performed at the times given in note 1 of table 3

2 cfu = colony forming units; \* indicates cfu per ml

3 Origin or typing collection no.; CCUG: Culture Collection University of Gothenburg, Sweden; – indicate a strain from "our own" culture collection that has not yet been typed at another culture collection

## Quality control of the test material

It is essential to have a homogeneous sample mixture and a uniform volume in all vials in order to allow comparison of all freeze-dried samples derived from one sample mixture. The volume was checked by weighing 2 to 3 % of the number of vials produced from the sample mixtures. The largest differences between vials were 8, 4 and 8 mg in mixture A, B and C, respectively. The largest accepted difference is 15 mg (3 %).

**Table 3** Contents (cfu) and measures of homogeneity ( $I_2$  and  $T$ , see reference 1) in relevant sample volumes for the various parameters in the samples

Analysis parameter <i>Method standard for analysis</i>	Sample <sup>1</sup>								
	A			B			C		
	cfu	$I_2$	T	cfu	$I_2$	T	cfu	$I_2$	T
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	21 <sup>b</sup>	0.8	1.4	24 <sup>c</sup>	1.4	1.6	65 <sup>a</sup>	0.4	1.2
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar. 44 °C according to SS 028167</i>	8 <sup>b</sup>	0.5	1.7	9	1.1	2.0	– <sup>2</sup>	–	–
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	10 <sup>b</sup>	1.4	2.1	7 <sup>c</sup>	0.6	1.7	–	–	–
Intestinal enterococci (MF) <i>m-Enterococcus Agar acc. to SS-EN ISO 7899-2:2000</i>	3 <sup>b</sup>	0.5	2.3	43 <sup>c</sup>	0.8	1.3	–	–	–
<i>Pseudomonas aeruginosa</i> (MF) <i>Pseudomonas Agar base with cetrimide and nalidixic acid according to SS-EN ISO 16266:2008</i>	–	–	–	34 <sup>c</sup>	0.7	1.4	12 <sup>a</sup>	1.0	1.7
Culturable microorg. 2d 37 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	22	1.5	1.7	64	0.6	1.2	41	1.1	1.4
Culturable microorg. 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	23	1.3	1.6	1	0.9	5.9	38	1.0	1.4

1 10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, analysed 22, 13 and 20 weeks ahead of the testing round for the sample A, B and C, respectively

2 Analysis of homogeneity was not performed on m-FC

a Determined for the volume 10 ml

b Determined for the volume 1 ml

c Determined for the volume 50 ml

– No target organism and thus no analysis

Table 3 presents the results from the organizer in the form of concentration means (cfu) and the measures ( $I_2$  and  $T$ ; see reference 1) used to assess homogeneity from duplicate analyses of 10 vials from each mixture the first time a mixture is used or duplicate analyses from 5 vials in a stability check when a mixture is used a second time. The results relate to the volume that was used for counting the colonies. The

criterion used for a sample mixture to be considered homogenous is that  $I_2$  and T *not simultaneously* are higher than 2. According to that criterion, all mixtures were homogeneous regarding the parameters that were about to be analysed.

## Processing of numerical results

Most histograms have "tails" in either or both directions, due to values that do not belong to a normal distribution. For drinking water, ten log transformation of results is seldom routine. With low concentrations as there, calculations are here instead performed after square root transformations of the results, which give the best normal distributions by decreasing the significance of the high deviating results. Very deviating values will still be present in most analyses and are identified as outliers (black bars). False negative results are presented with white bars in the histograms.

Outliers are identified by use of Grubbs' test according to a modification by Kelly [3]. A level of 1 % is set as the risk to incorrectly assess a result as being an outlier. Although the method is objective, there is a prerequisite that the results are normally distributed in order to obtain correct outliers at the 1 % level. A zero result that is a low outlier is considered a false negative result. In special situations, e.g. when many zero results are reported and in some borderline cases, subjective adjustments will be made based on the knowledge of the sample mixture's content in order to set the right limits. False results and outliers are not included in the calculations of mean values and measures of distribution.

The coefficient of variation (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.

The calculation of uncertainty of measurement of the assigned value is described in the scheme protocol [1]. The assigned value for an analysis is here calculated from the square root transformed results and is the square root of "Mean" in Annex A. It is there denoted as  $mv$ . Hence, also the measurement uncertainty will be expressed as a square root value. The standard uncertainty of measurement ( $u$ ) correspond to the standard deviation of the assigned value ( $s$ ) divided by the number of results squared-root transformed, i.e.:  $u = s/\sqrt{n_{mv}}$  where  $n_{mv}$  is the number of results in annex A, except the deviating ones. Here is the relative uncertainty ( $u_{rel}$ ) used and expressed as per cent after division by the mean value  $mv$  and multiplication by 100.

More about result processing and recommendations on follow-up work are given in the scheme protocol [1]. A PDF of that document is available on the website <https://www2.slv.se/absint>.



## References

1. Anonymous 2018. Scheme protocol, Microbiology, Drinking water & Food, 5<sup>th</sup> ed. Swedish Food Agency (formerly National Food Agency), Sweden.
2. Peterz, M., Steneryd, A.-C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *J. Appl. Bacteriol.* 74:143-148.
3. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Chem.* 73:58-64.
4. Anonymous 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Official Journal of the European Communities.* 5.12.98, L 330/32-54 (*national translations available*).
5. Standard Methods for the Examination of Water and Wastewater, <http://www.standardmethods.org/>
6. Anonymous 2015. Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption. *Official Journal of the European Union.* 7.10.2015, L 260/6-17 (*national translations available*).





Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria ("rapid" MPN)			<i>E. coli</i> ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
8329	1 2 3	-	-	-	-	-	-	-	-	-	-	-	1796	43	345	717	19	0	
8380	2 1 3	-	-	-	2000	33	310	-	-	-	800	17	0	1600	19	350	610	19	0
8435	2 3 1	-	-	-	1950	13	80	480	13	89	650	13	0	-	-	-	-	-	-
8569	1 3 2	1360	15	330	1360	15	330	-	-	-	640	15	0	1990	36	310	800	12	0
8626	2 3 1	2070	7	370	1150	7	370	-	-	-	1150	7	0	-	-	-	-	-	-
8628	1 2 3	-	-	-	2300	<300	<300	900	<300	<300	900	<300	<300	-	-	-	-	-	-
8663	1 2 3	2300	540	16	2300	380	14	760	250	5	2100	0	8	2400	520	19	1100	0	15
8742	3 1 2	-	-	-	1100	18	130	-	-	-	570	10	<1	-	-	-	-	-	-
8766	3 1 2	1827	25	218	1827	25	218	-	-	-	909	25	0	2012	25	218	745	25	0
8862	1 2 3	1763	26	464	1763	26	264	-	-	-	918	11	0	1379	62	359	1025	29	0
8891	2 1 3	-	-	-	400	10	<1	-	-	-	-	-	-	-	-	-	-	-	-
8898	2 1 3	1829	34	486	1829	34	391	-	-	-	1000	10	0	1790	21	365	769	9	0
8955	2 3 1	-	-	-	1200	39	210	700	11	110	900	17	0	1400	30	340	920	22	0
9002	1 2 3	-	-	-	29	21	2	-	-	-	5	0	0	-	-	-	-	-	-
9051	1 2 3	-	-	-	1368	8	79	-	-	-	729	8	0	1733	9	119	1046	9	<1
9306	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	1787	11	425	847	11	0
9408	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	2419	20	411	687	20	<1
9436	2 1 3	1482	12	485	1482	12	345	618	4	309	809	12	0	1938	19	566	1098	19	0
9441	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	2380	24	560	1640	14	0
9524	3 2 1	-	-	-	2600	7	280	-	-	-	1000	7	<1	2086	14	313	1248	14	<1
9736	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	1226	71	330	582	42	0
9899	3 1 2	1648	13	410	1648	13	410	-	-	-	881	13	0	2356	42	423	967	14	0
9903	2 1 3	2045	44	556	2045	44	320	1550	10	272	900	10	0	-	-	-	-	-	-

n	42	42	42	67	66	67	26	25	26	67	65	67	63	63	63	63	63	63	65
Min	733	0	16	29	0	0	400	0	0	5	0	0	870	2	0	0	0	0	0
Max	12700	540	10800	2700	380	544	1600	250	500	8400	25	8	28000	520	566	1640	42	15	
Median	1650	24	391	1631	21.5	267	710	10	130	809.5	12	0	1793	19.5	359	866	16	0	
Mean				1587	20	232				857	12	0	1788	20	343	899	15	0	
CV (%)				15	32	33				17	25	-	11	27	17	11	25	-	
False positive				0	0	0				0	0	1	0	0	0	0	0	1	
False negative				0	2	7				0	5	0	0	0	1	1	2	0	
Outliers, low				1	0	0				4	0	0	0	0	1	1	0	0	
Outliers, high				0	2	0				1	0	0	1	1	0	0	0	0	
Low limit OK	733	0	16	400	1	2	400	0	0	400	1	0	870	2	70	582	2	0	
High limit OK	1E+05	540	10800	2700	50	544	1600	250	500	2100	25	0	2500	71	566	1640	42	0	

mv ( $\sqrt{\text{Mean}}$ )				39.836	4.498	15.218				29.266	3.449	0.000	42.290	4.510	18.531	29.977	3.912	0.000
s ( $CV \cdot mv/100$ )				6.102	1.439	4.990				4.924	0.867	0.000	4.767	1.217	3.231	3.205	0.965	0.000
$u_{rel,mv}$ (%) ( $100 \cdot s / \sqrt{n_{mv}} / mv$ )				1.9	4.1	4.2				2.1	3.2		1.4	3.4	2.2	1.4	3.2	
x ( $\sqrt{\text{Result}}$ )																		
z ( $(x-mv)/s$ )																		

# cfu/ml

Susp. intestinal enterococci (MF)			Intestinal enterococci			Susp. <i>Pseudomonas aeruginosa</i> (MF)			<i>Pseudomonas aeruginosa</i>			Total plate count 22 °C, 3 days #			Total plate count 36±2 °C, 2 days #			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
367	12	2773	367	12	0	-	-	-	0	31	81	25	1	45	31	82	40	8329
-	-	-	290	62	0	-	-	-	0	35	100	40	3	33	26	74	36	8380
-	-	-	300	55	0	0	21	74	0	7	74	21	2	60	24	56	40	8435
310	63	2100	310	63	2100	-	-	-	-	-	-	28	0	56	-	-	-	8569
-	-	-	-	-	-	-	-	-	-	-	-	38	0	9	-	-	-	8626
-	-	-	400	73	0	-	-	-	0	27	140	26	3	38	43	84	44	8628
510	300	70	510	0	16	0	77	35	0	77	35	20	39	1	31	42	73	8663
-	-	-	-	-	-	-	-	-	-	-	-	31	520	42	32	100	42	8742
350	66	1800	350	43	0	690	33	91	690	33	91	28	4	36	59	73	36	8766
373	73	3900	373	0	0	-	-	-	-	-	-	29	4	38	35	50	38	8862
-	-	-	-	-	-	-	-	-	-	-	-	40	1	40	-	-	-	8891
332	80	0	332	80	0	0	48	78	0	48	78	33	1	39	33	63	46	8898
350	91	3700	350	90	0	-	-	-	0	48	93	34	5	34	32	56	32	8955
-	-	-	32	22	0	-	-	-	-	-	-	50	2	120	-	-	-	9002
-	-	-	252	90	0	-	-	-	0	20	44	14	4	21	22	52	21	9051
-	-	-	-	-	-	-	-	-	-	-	-	18	2	41	28	61	40	9306
-	-	-	390	0	0	-	-	-	0	48	94	23	1	26	26	75	41	9408
327	56	3624	327	25	0	509	41	69	0	41	69	21	2	34	31	77	50	9436
344	101	0	-	-	-	-	-	-	-	-	-	29	2	41	25	60	46	9441
-	-	-	355	54	<1	-	-	-	-	-	-	36	1	38	28	63	46	9524
325	117	0	325	117	0	0	43	111	0	43	111	16	4	37	25	55	30	9736
338	71	0	338	71	0	0	43	93	0	43	93	30	3	42	30	57	38	9899
342	91	13	342	44	0	0	60	128	0	60	128	33	1	40	35	67	41	9903
48	48	45	70	70	70	32	32	32	58	58	59	85	85	85	71	71	71	n
0	0	0	32	0	0	0	0	35	0	0	10	2	0	1	15	11	21	Min
518	300	4100	518	159	2100	690	77	2120	2600	120	150	1020	700	8300	200	13273	162	Max
365	80	70	355.5	66	0	0	43	85	0	43	82	26	2	38	27.5	60	38	Median
			359	56	0				0	41	83	26	2	38	28	57	38	Mean
			9	31	-				-	16	17	14	56	9	11	14	9	CV (%)
			0	0	4				4	0	0	0	0	0	0	0	0	False pos.
			0	8	0				0	1	0	0	0	0	0	0	0	False neg.
			2	0	0				0	1	1	1	0	2	0	0	0	Outliers <
			0	0	0				0	1	0	3	6	3	3	2	2	Outliers >
0	0	0	233	3	0	0	0	35	0	18	26	14	0	21	15	11	21	Low limit
518	300	4100	518	159	0	690	77	2120	0	100	150	50	10	60	49	86	55	High limit
			18.956	7.512	0.000				0.000	6.435	9.105	5.129	1.261	6.157	5.263	7.579	6.191	mv
			1.715	2.354	0.000				0.000	1.058	1.503	0.696	0.701	0.568	0.565	1.045	0.563	s
			1.1	4.0					2.2	2.2		1.5	6.3	1.0	1.3	1.7	1.1	u <sub>rel,mv</sub> (%)
																		x
																		z

# cfu/ml





Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria ("rapid" MPN)			<i>E. coli</i> ("rapid" MPN)			
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
9002	3 1 2				-4.000	0.058	-2.767				-4.000		0.000							
9051	2 1 3				-0.467	-1.160	-1.269				-0.460	-0.716	0.000	-0.139	-1.241	-2.360	0.738	-0.945	0.000	
9306	1 3 2													-0.004	-0.980	0.645	-0.273	-0.616	0.000	
9408	1 3 2													1.446	-0.031	0.539	-1.175	0.581	0.000	
9436	2 3 1				-0.220	-0.719	0.673				-0.167	0.018	0.000	0.363	-0.124	1.628	0.986	0.464	0.000	
9441	1 3 2													1.362	0.320	1.589	3.282	-0.176	0.000	
9524	1 3 2				1.828	-1.287	0.304				0.479	-0.926	0.000	0.710	-0.631	-0.260	1.669	-0.176	0.000	
9736	2 3 1													-1.526	3.219	-0.113	-1.826	2.662	0.000	
9899	1 2 3				0.125	-0.620	1.008				0.084	0.181	0.000	1.311	1.620	0.630	0.349	-0.176	0.000	
9903	3 2 1				0.883	1.483	0.535				0.149	-0.330	0.000							

n		0	0	0	67	64	60	0	0	0	67	60	66	63	63	62	62	61	64
Min					-4.000	-2.431	-2.767				-4.000	-2.824	0.000	-2.684	-2.544	-4.000	-4.000	-2.588	0.000
Max					1.987	4.000	1.624				4.000	1.789	0.000	4.000	4.000	1.628	3.282	2.662	0.000
Median					0.054	0.133	0.225				-0.199	0.018	0.000	0.019	-0.031	0.092	-0.171	0.092	0.000
Mean					-0.060	0.122	0.000				-0.179	0.000	0.000	0.063	0.063	-0.065	-0.065	0.000	0.000
SD					1.106	1.198	1.000				1.450	1.000	0.000	1.113	1.113	1.114	1.114	1.000	0.000
z<-3					2	0	0				4	0	0	0	0	2	1	0	0
-3≤z<-2					2	2	4				0	3	0	3	1	3	0	1	0
-2<z≤3					0	0	0				2	0	0	0	1	0	1	2	0
z>3					0	2	0				2	0	0	1	2	0	1	0	0

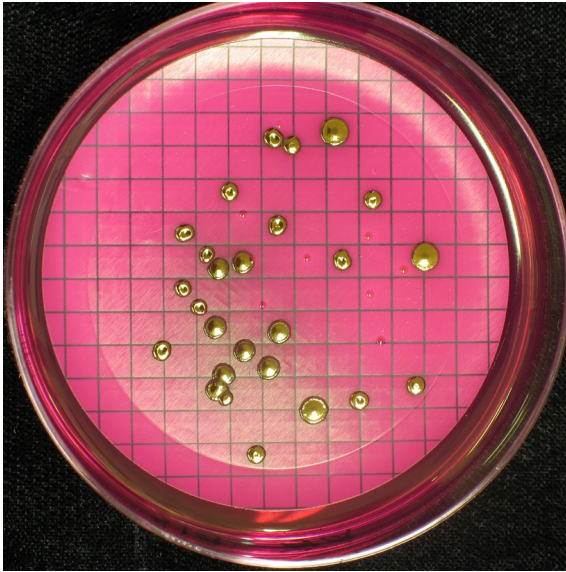


Susp. intestinal enterococci (MF)			Intestinal enterococci			Susp. <i>Pseudomonas aeruginosa</i> (MF)			<i>Pseudomonas aeruginosa</i>			Total plate count 22 °C, 3 days			Total plate count 36±2 °C, 2 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
			-4.000	-1.199	0.000							2.789	0.218	4.000				9002
			-1.797	0.839	0.000				0.000	-1.855	-1.644	-1.994	1.054	-2.772	-1.013	-0.353	-2.856	9051
			0.462		0.000				0.000	0.466	0.393	-1.274	0.218	0.434	0.050	0.221	0.238	9306
			-0.509	-1.067	0.000				0.000	-0.030	-0.531	-0.479	-0.373	-1.863	-0.290	1.035	0.377	9408
												0.367	0.218	0.434	0.539	1.145	1.563	9436
												1.251	-0.373	0.013	-0.465	0.160	1.051	9441
												0.000	0.116	0.952	0.050	0.343	1.051	9524
												-1.623	1.054	-0.130	-0.465	-0.156	-1.267	9736
												0.000	0.116	0.358	0.379	-0.028	-0.047	9899
												0.000	1.240	1.469	1.155	0.580	0.377	9903

0	0	0	70	62	66	0	0	0	54	57	59	85	85	85	71	71	71	n
			-4.000	-2.456	0.000				0.000	-3.582	-3.954	-4.000	-1.800	-4.000	-2.459	-4.000	-2.856	Min
			2.218	2.165	0.000				0.000	4.000	2.091	4.000	4.000	4.000	4.000	4.000	4.000	Max
									0.000	0.116	-0.033	-0.044	0.218	0.013	0.050	0.160	0.096	Median
									0.000	0.007	-0.067	0.094	0.282	0.047	0.169	0.090	0.113	Mean
									0.000	1.216	1.117	1.306	1.411	1.375	1.270	1.122	1.190	SD
			2	0	0				0	1	1	1	0	2	0	1	0	Summa
			1	1	0				0	1	2	0	0	2	2	3	3	17
			2	1	0				0	1	1	2	1	2	1	1	3	34
			0	0	0				0	2	0	3	6	3	4	1	2	21
																		29

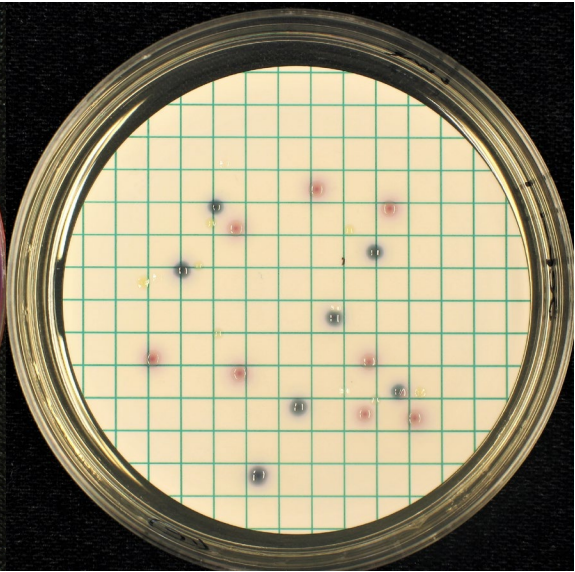
Mixture A

m-Endo Agar LES, 37 °C



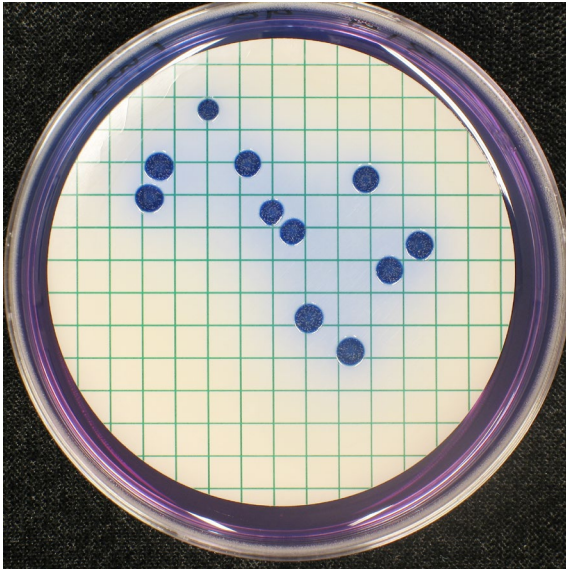
1 ml

Chromocult Coliform Agar, 37 °C



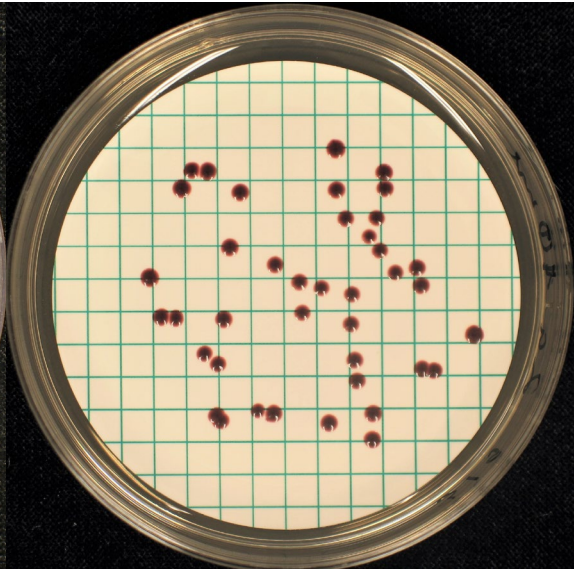
1 ml

m-FC Agar, 44 °C



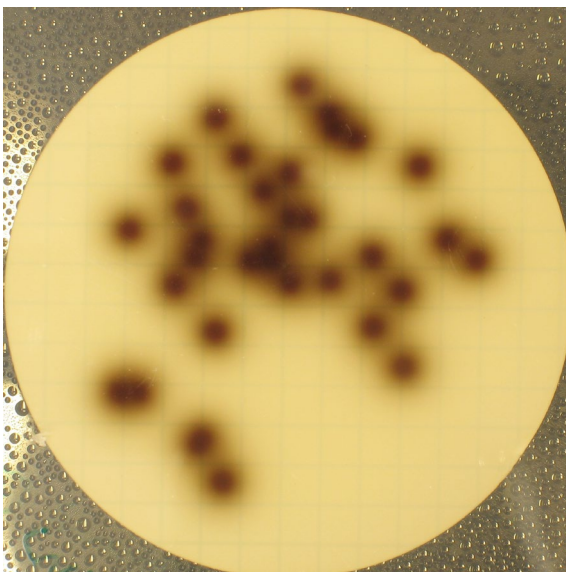
1 ml

m-Enterococcus Agar, 37 °C



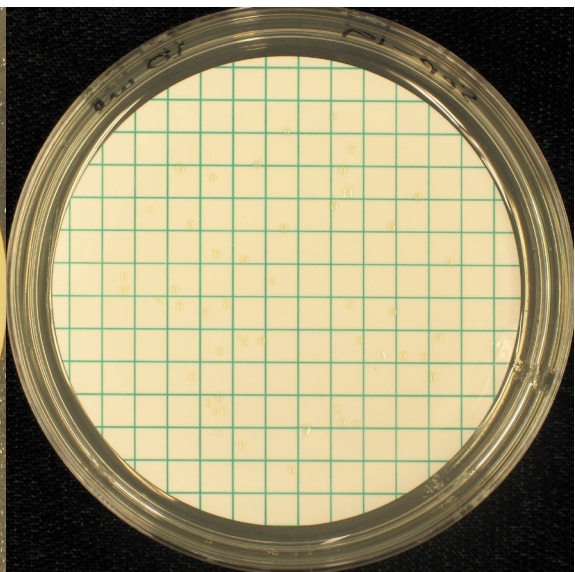
10 ml, 2 days

Bile Esculin Azide Agar, 44 °C



10 ml, 2 hours (from beneath)

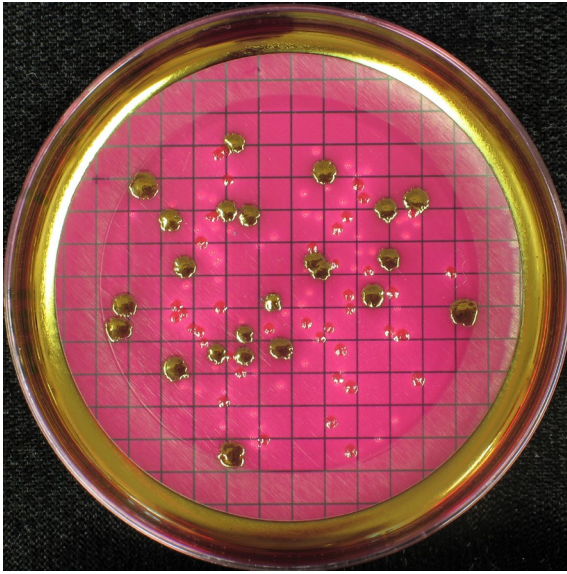
m-Pseudomonas CN Agar, 37 °C



10 ml, 2 days

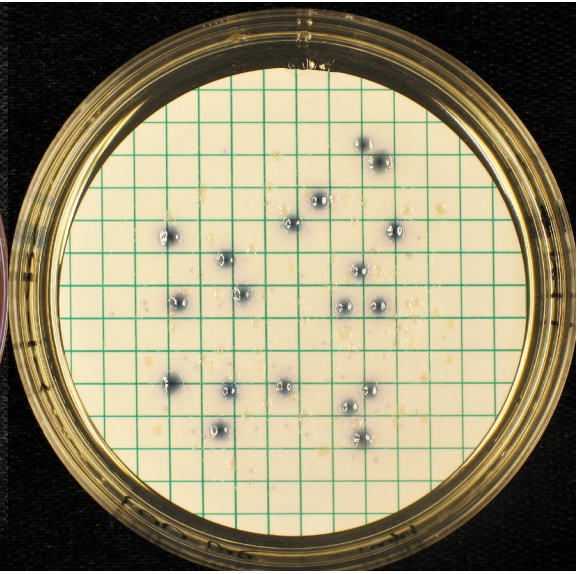
# Mixture B

m-Endo Agar LES, 37 °C



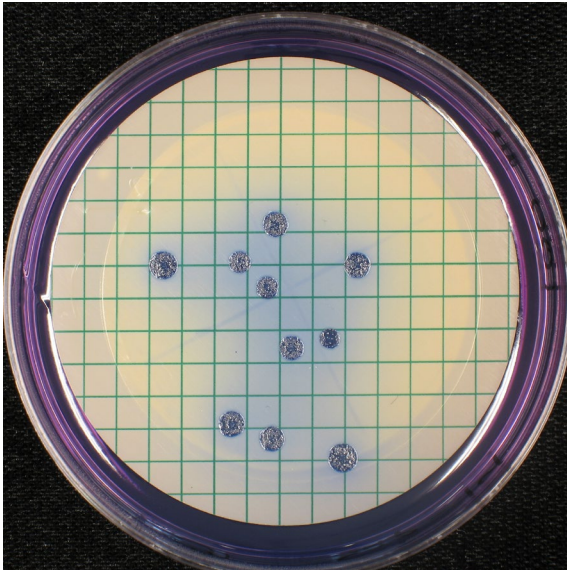
100 ml

Chromocult Coliform Agar, 37 °C



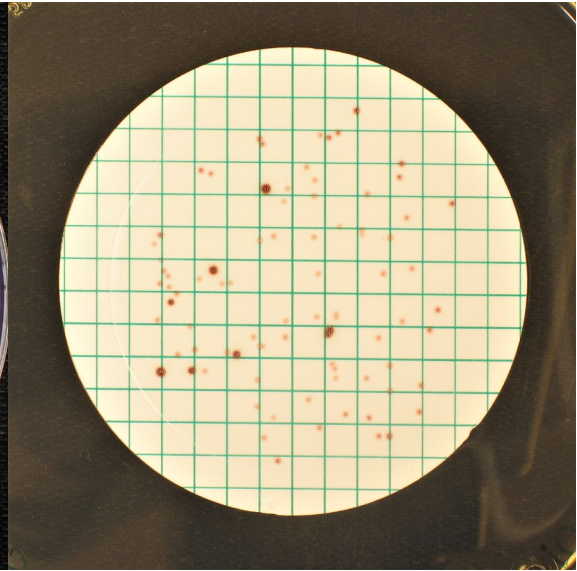
100 ml

m-FC Agar, 44 °C



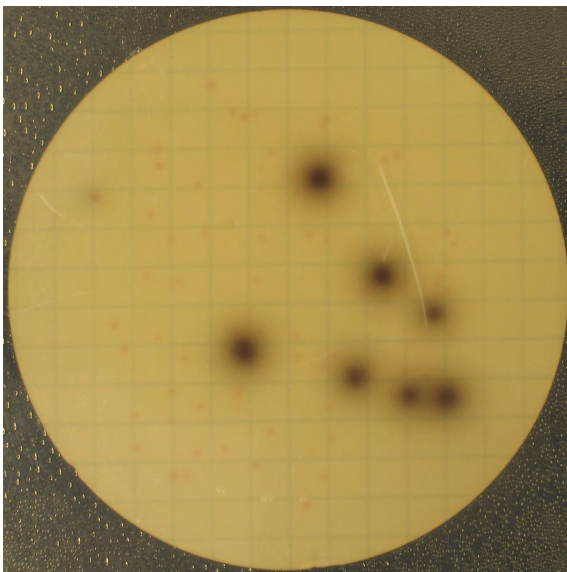
100 ml

m-Enterococcus Agar, 37 °C



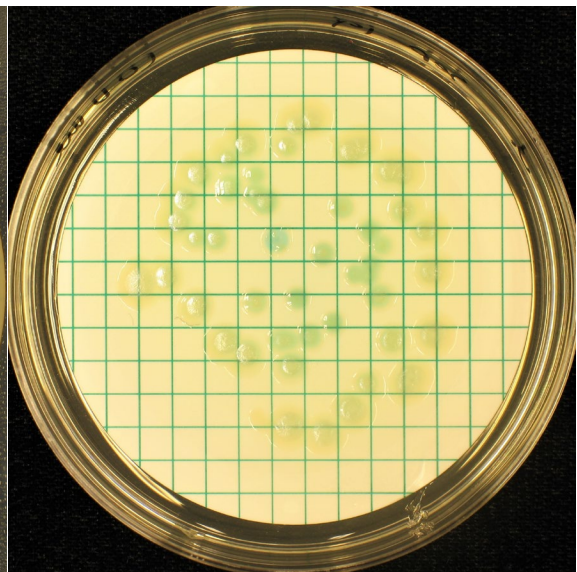
100 ml, 2 days

Bile Esculin Azide Agar, 44 °C



100 ml, 2 hours (from beneath)

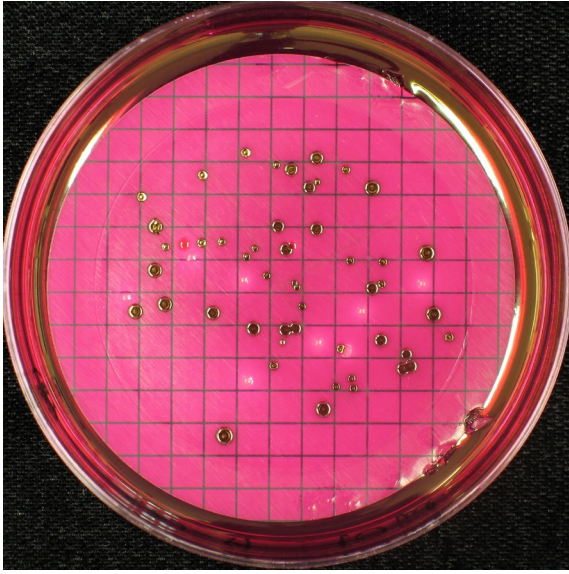
m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

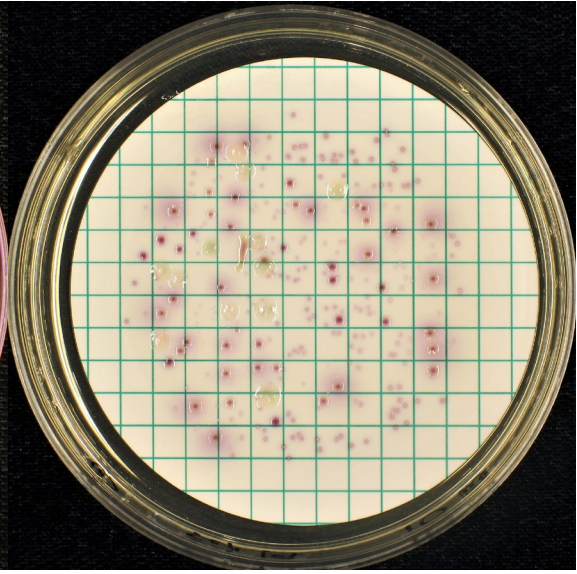
# Mixture C

m-Endo Agar LES, 37 °C



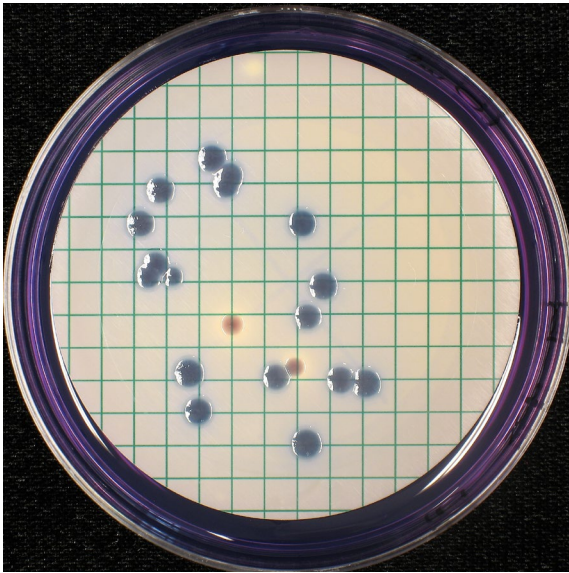
10 ml

Chromocult Coliform Agar, 37 °C



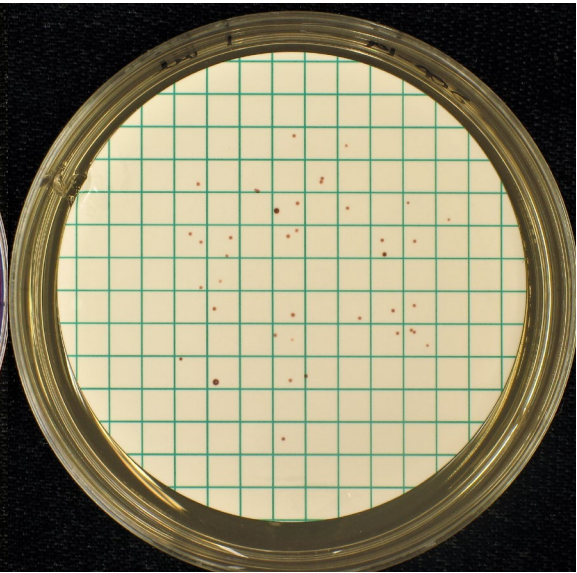
10 ml

m-FC Agar, 44 °C



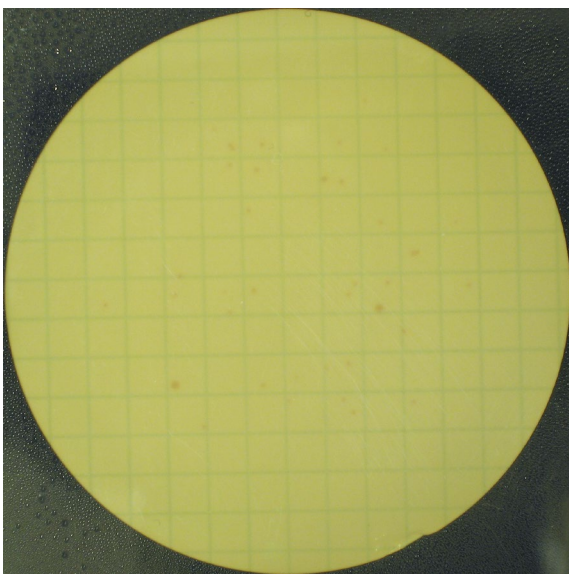
10 ml

m-Enterococcus Agar, 37 °C



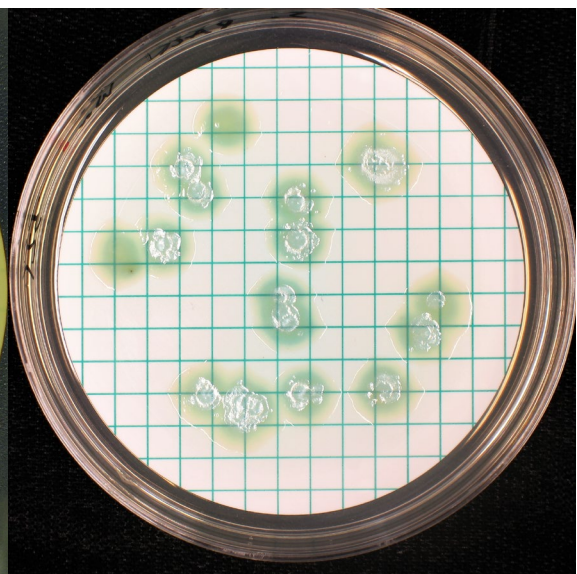
1 ml

Bile Esculin Azide Agar, 44 °C



1 ml, 2 days (from beneath)

m-Pseudomonas CN Agar, 37 °C



10 ml, 2 days

### **PT reports published 2018**

Proficiency Testing – Food Microbiology, January 2018, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, March 2018, by Tommy Šlapokas

Proficiency Testing – Food Microbiology, April 2018, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, September 2018, by Tommy Šlapokas

Proficiency Testing – Food Microbiology, October 2018, by Jonas Ilbäck

### **PT reports published 2019**

Proficiency Testing – Food Microbiology, January 2019, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, March 2019, by Tommy Šlapokas

Proficiency Testing – Food Microbiology, April 2019, by Jonas Ilbäck

## **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 laboratories carry out some form of internal quality assurance, but their analytical work also has 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 proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The laboratories report their results to the organiser that evaluates them and compiles them in a report.

### **The National Food Agency's PT program offers**

- External and independent evaluation of laboratories analytical competence.
- Improved knowledge of analytical methods with respect to various types of organisms.
- Expert support.
- Tool for inspections regarding accreditation.
- Free extra material for follow-up analyses

For more information, visit our website: <https://www2.slv.se/absint>

### **The National Food Agency's reference material**

As a complement to the proficiency testing but without specific accreditation, National Food Agency also produces reference material (RM) for internal quality control: a total of 8 RM for food and drinking water microbiological analyses, including pathogens, are available.

Information available on our website: <https://www.livsmedelsverket.se/en/RM-micro>