

## Drinking Water Microbiology

March 2021

Linnea Blom & Tommy Šlapokas



*Edition*

Version 1 (2021-06-08)

*Editor in chief*

Maria Sitell, Head of Biology department, Swedish Food Agency

*Responsible for the scheme*

Tommy Šlapokas, Microbiologist, Biology department, Swedish Food Agency

*Responsible for the report*

Linnea Blom, Laboratory Engineer, Biology department, Swedish Food Agency

PT March 2021 is registered as no. 2021/00683 at the Swedish Food Agency, Uppsala

*Proficiency testing*

# Drinking water Microbiology

March 2021

## 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

**Slow-growing bacteria** 7 days incubation at  $22\pm 2$  °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)
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

# Contents

---

<b>Abbreviations and explanations</b> .....	2
<b>Contents</b> .....	3
<b>General information on results evaluation</b> .....	4
<b>Results of the PT round</b> .....	4
- General outcome .....	4
- Coliform bacteria (MF) .....	6
- Suspected thermotolerant coliform bacteria (MF) .....	7
- <i>Escherichia coli</i> (MF) .....	9
Coliform bacteria and <i>E. coli</i> (rapid method, MPN) .....	10
- Presumptive and confirmed <i>Clostridium perfringens</i> (MF) .....	13
- Moulds and yeasts (MF) .....	15
- Actinomycetes (MF) .....	17
- Culturable microorganisms 22 °C, 3 days .....	18
- Slow-growing bacteria 22 °C, 7 days .....	20
<b>Outcome of the results and laboratory assessment</b> .....	23
- General information about reported results .....	23
- Base for assessment of the performance .....	23
- Mixed up results and other practical errors .....	23
- Z-scores, box plots and deviating results for each laboratory .....	23
<b>Test material, quality control and processing of data</b> .....	27
- Description of the test material .....	27
- Quality control of the test material .....	28
- Processing of numerical results .....	29
<b>References</b> .....	30
<b>Annex A – All reported results</b> .....	32
<b>Annex B – Z-scores of the results</b> .....	36
<b>Annex C – Photo example of colony appearance on some media</b> .....	38

## 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

---

Test items were dispatched to 75 laboratories, 35 in Sweden, 35 in other Nordic countries (Faeroe Islands, Greenland and Åland included), two more from EU, and two from the rest of Europe and one from outside Europe. Results were reported by 74 laboratories.

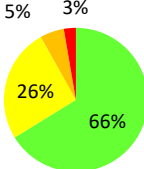
The percentages of false results and outliers are compiled in **table 1**.

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 in tables 1 and 3). 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 shaded rows are not assessed.

Sample	A			B			C		
Percentage of laboratories with									
No. of evaluable results	481			469			481		
No. of deviating results *	36 (7 %)			28 (6 %)			36 (7 %)		
Microorganisms	<i>Klebsiella pneumoniae</i> <i>Acremonium strictum</i> <i>Hanseniaspora uvarum</i> <i>Sphingomonas</i> sp. <i>Staphylococcus warneri</i>			<i>Citrobacter freundii</i> <i>Klebsiella oxytoca</i> <i>Clostridium perfringens</i> <i>Streptomyces</i> sp. <i>Staphylococcus saprophyticus</i>			<i>Escherichia coli</i> <i>Enterobacter cloacae</i> <i>Clostridium bifermentans</i> <i>Sphingomonas</i> sp. <i>Cladosporium cladosporioides</i>		
	Analysis	Target org.	F%	X%	Target org.	F%	X%	Target org.	F%
Coliform bacteria (MF)	<i>K. pneumoniae</i>	0	4	<i>C. freundii</i> <i>K. oxytoca</i>	0	8	<i>E. coli</i> <i>E. cloacae</i>	0	2
Susp. thermotolerant coliform bact. (MF)	<i>K. pneumoniae</i>	–	–	–	–	–	<i>E. coli</i> { <i>E. cloacae</i> }	–	–
<i>E. coli</i> (MF)	–	2	–	–	6	–	<i>E. coli</i>	6	8
Coliform bacteria (rapid method)	<i>K. pneumoniae</i>	0	10	<i>C. freundii</i> <i>K. oxytoca</i>	0	4	<i>E. coli</i> <i>E. cloacae</i>	0	6
<i>E. coli</i> (rapid meth.)	–	0	–	–	2	–	<i>E. coli</i>	2	2
Presumptive <i>C. perfringens</i> (MF)	–	5	–	<i>C. perfringens</i>	0	8	<i>C. bifermentans</i>	11	0
<i>C. perfringens</i> (MF)	–	8	–	<i>C. perfringens</i>	4	4	[ <i>C. bifermentans</i> ]	19	–
Actinomycetes (MF) 25 °C	–	3	–	<i>Streptomyces</i> sp.	7	3	–	0	–
Moulds (MF) 25 °C	<i>A. strictum</i>	34	11	–	11	0	<i>C. cladosporioides</i>	13	3
Yeasts (MF) 25 °C	<i>H. uvarum</i>	8	5	–	3	–	–	5	–
Culturable micro-organisms (total count), 3 days 22 °C	<i>K. pneumoniae</i> ( <i>S. warneri</i> ) { <i>Sphingo. sp.</i> }	0	0	<i>S. saprophyticus</i> ( <i>C. freundii</i> ) ( <i>K. oxytoca</i> )	1	3	<i>E. cloacae</i> ( <i>E. coli</i> ) ( <i>Sphingo. sp.</i> )	1	1
Slow-growing bacteria (total count), 7 days 22 °C	<i>Sphingo. sp.</i> <i>K. pneumoniae</i> ( <i>S. warneri</i> )	0	3	<i>S. saprophyticus</i> ( <i>C. freundii</i> ) ( <i>K. oxytoca</i> )	0	5	<i>E. cloacae</i> ( <i>E. coli</i> ) ( <i>Sphingo. sp.</i> )	0	10

\* In total 41 of 74 laboratories (55 %) 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

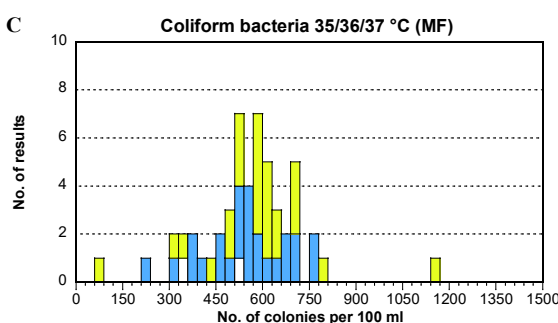
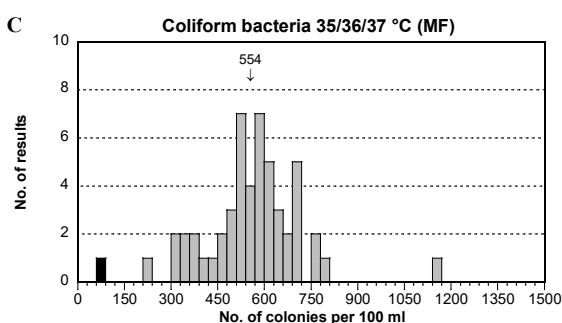
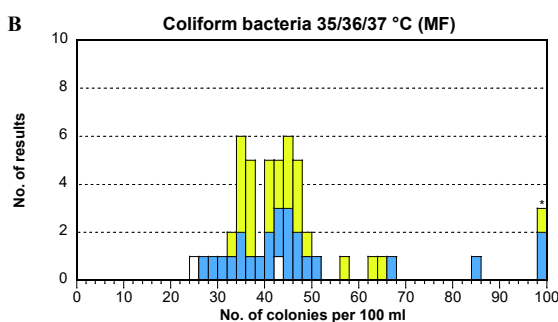
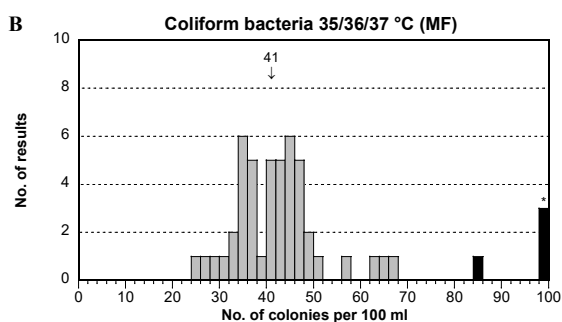
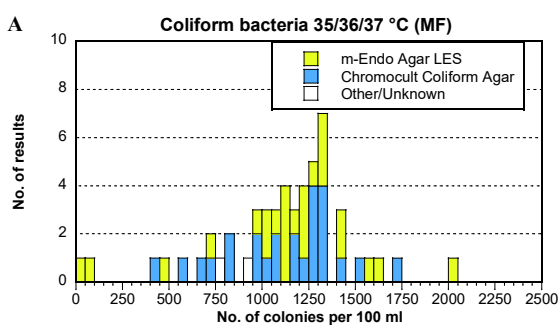
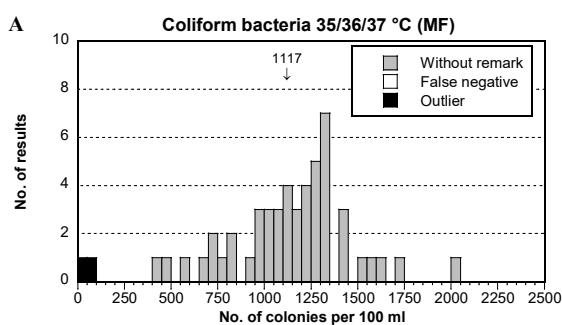
## Coliform bacteria (MF)

The exact same number of laboratories used CCA and LES. One of the two laboratories within the group Other/Unknown used trypton glucose yeast extract agar (TGE) and incubated at room temperature for seven days. The other laboratory reported the use of (m-)Lactose Glucuronide Agar (MLGA).

As often before, CCA gave lower average result than LES, at least in samples A and C. The results for CCA are the same as those reported as obtained by the use of the standard EN ISO 9308-1:2014 (with or without Amendment A1:2017).

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

Medium	N	A					B					C							
		n	M <sub>v</sub>	CV	F	<	>	n	M <sub>v</sub>	CV	F	<	>	n	M <sub>v</sub>	CV	F	<	>
<b>Total</b>	<b>52</b>	<b>50</b>	<b>1117</b>	<b>14</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>46</b>	<b>41</b>	<b>11</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>51</b>	<b>554</b>	<b>13</b>	<b>0</b>	<b>1</b>	<b>0</b>
m-Endo Agar LES	25	23	1185	13	0	2	0	24	43	10	0	0	1	24	587	13	0	1	0
Chromocult C Agar	25	25	1081	15	0	0	0	20	40	11	0	0	3	25	534	13	0	0	0
Other/Unknown	2	2	–	–	0	0	0	2	–	–	0	0	0	2	–	–	0	0	0





### Sample A

- Only the coliform bacteria *Klebsiella pneumoniae* was present. This strain appeared with for coliform bacteria, typical colonies on MF media at 35/36/37 °C, i.e. with metallic sheen on LES and pink on CCA.
- The distribution of the results was fairly good with a small dispersion (CV). Two low outliers were present. One of these is from a laboratory that has probably mixed up their samples, given that they reported a low outlier in sample A and a high outlier in sample B. The other laboratory with a low outlier reported a low outlier in sample C as well.
- The average result for coliform bacteria was somewhat higher with rapid methods (Colilert®; page 10) than for the MF-methods, 1341 versus 1117 cfu /100 ml, indicating that the strain of *K. pneumoniae* was not detected to the full extent by the MF-methods.

### Sample B

- Two strains of coliform bacteria, *C. freundii* and *K. oxytoca*, were present in the sample. These strains appeared with typical colonies at 35/36/37 °C, i.e. with metallic sheen on LES and pink on CCA. There were also some other small pink colonies present on CCA, making them a bit more difficult to identify and count the coliform bacteria.
- Despite the background flora, the distribution of the accepted results was good and the dispersion was small. Four high outliers were present, of which one result could be due to the background flora being included.

### Sample C

- Two strains of coliform bacteria were included, of which one was *E. coli* and the other *Enterobacter cloacae*. Both show typical appearance on MF media at 35/36/37 °C, metallic sheen on LES and blue and pink, respectively, on CCA.
- The distribution of the results was good with a small dispersion. One low outlier was present.
- As in sample A, there was a tendency for somewhat higher average results with rapid methods (Colilert®; page 10); 652 versus 554 cfu / 100 ml compared to the MF-methods.

## **Suspected thermotolerant coliform bacteria (MF)**

---

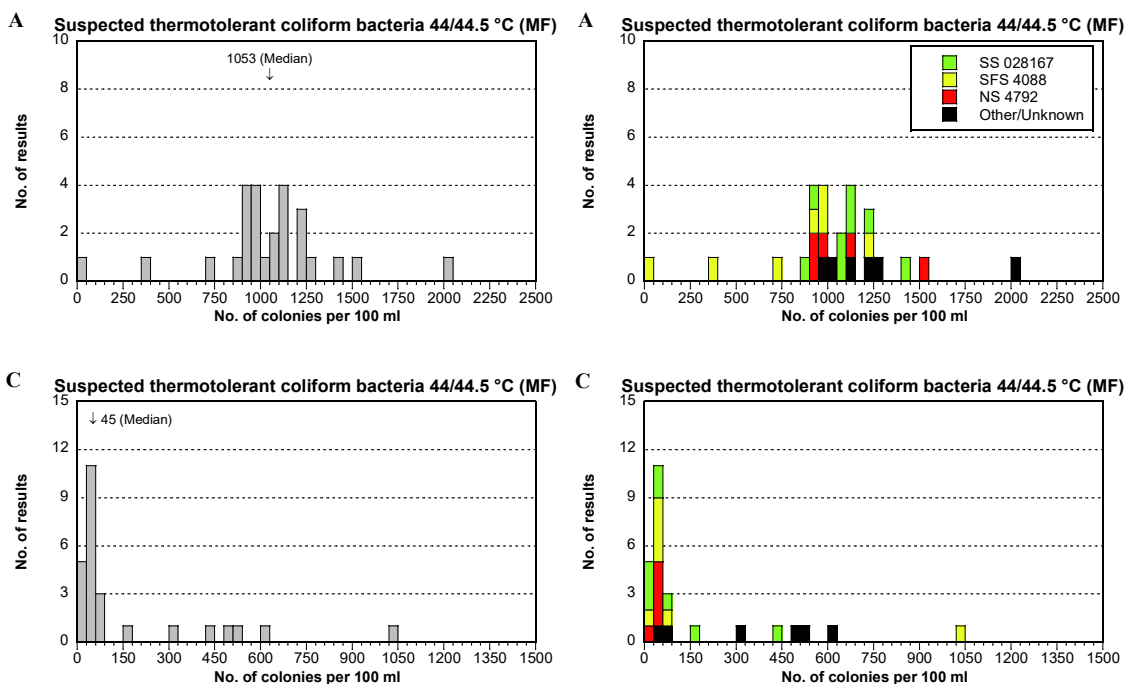
No evaluation in relation to performance is done for what is called suspected (not confirmed) colonies of a parameter. Therefore, no identification of outliers is done. The *medians* are then more robust than the means and are given in the table and in the histograms. **Thus, the parameter is not included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

The primary growth media used at 44 or 44.5 °C to identify suspected thermotolerant coliform bacteria is m-FC. Two laboratories in the group Other/Unknown have stated methods where the primary media are incubated at 35/36/37 °C, and where 44 °C is used only for confirmation. This is not the intention of the parameter suspected

thermotolerant coliform bacteria according to the definition in the instruction and on the website for the program. Instead, it is the typical colonies appearing on the membrane filter at 44/44.5 °C that should be reported. Some laboratories have reported incubation at 44 / 44.5 °C, but it is doubtful whether this is accurate.

Standard, Method	N	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
<b>Total</b>	<b>26</b>	<b>26</b>	<b>1053</b>	-	-	-	<b>26</b>	<b>0</b>	-	-	-	<b>26</b>	<b>45</b>	-	-	-
SS 028167	8	8	1108	-	-	-	8	0	-	-	-	8	41	-	-	-
SFS 4088	7	7	900	-	-	-	7	0	-	-	-	7	42	-	-	-
NS 4792	5	5	990	-	-	-	5	0	-	-	-	5	34	-	-	-
Other/Unknown	6	6	1150	-	-	-	6	23	-	-	-	6	399	-	-	-

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



### Sample A

- *K. pneumoniae* was the only coliform bacterium in the sample. It appears as a typical suspected thermotolerant coliform bacterium with blue colonies on m-FC agar at 44/44.5 °C.

### Sample B

- There were no thermotolerant coliform bacteria in the sample.
- Five laboratories reported results that were not zero cfu per 100 ml.

### Sample C

- 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 on m-FC at 44/44.5 °C. The strain of *E. cloacae* can also sometimes grow

as a (suspected) thermotolerant coliform bacterium with small blue colonies on m-FC, especially when the temperature does not fully reach 44 °C.

### ***Escherichia coli* (MF)**

Confirmation is required to identify and quantify *E. coli* 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 considered as a confirmed *E. coli*.

The primary growth media are CCA and LES that are incubated at 35/36/37 °C and m-FC that is incubated 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 35/36/37 °C on CCA. For the standards from the Nordic countries (SS, SFS and NS) the majority of the results are from 35/36/37 °C on LES but some are also from 44/44.5 °C on m-FC.

Only sample C contained *E. coli* and when comparing differences among the methods, the Finnish standard gave higher average result than those based on other standards and LES had higher recovery than CCA.

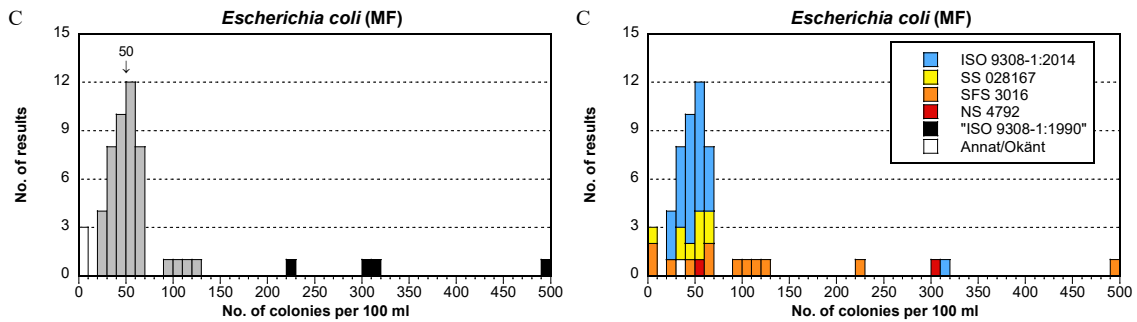
#### *All results*

Origin & Standard	N#	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	<b>53</b>	<b>52</b>	<b>0</b>	<b>-</b>	<b>1</b>	<b>-</b>	<b>-</b>	<b>48</b>	<b>0</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>-</b>	<b>46</b>	<b>50</b>	<b>20</b>	<b>3</b>	<b>0</b>	<b>4</b>
<i>Colony origin</i>																			
36 ± 2 °C	41	41	0	-	0	-	-	37	0	-	2	-	-	37	48	21	2	0	2
44/44.5 °C	5	5	0	-	0	-	-	5	0	-	0	-	-	3	-	-	0	0	2
36 ± 2 & 44/44.5 °C	7	6	0	-	1	-	-	6	0	-	1	-	-	6	59	16	1	0	0
<i>Standard</i>																			
ISO 9308-1:2014	29	29	0	-	0	-	-	25	0	-	2	-	-	28	45	14	0	0	1
SS 028167	9	9	0	-	0	-	-	9	0	-	0	-	-	8	49	11	1	0	0
SFS 3016 (4088)	12	12	0	-	0	-	-	11	0	-	1	-	-	8	73	27	2	0	2
"ISO 9308-1:1990"	2	1	-	-	1	-	-	2	-	-	0	-	-	1	-	-	0	0	1
Other/Unknown	1	1	-	-	0	-	-	1	-	-	0	-	-	1	-	-	0	0	0

#### *Results from the analysis of "coliform bacteria" MF at 35/36/37 °C*

Medium	N#	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	<b>43</b>	<b>43</b>	<b>0</b>	<b>-</b>	<b>0</b>	<b>-</b>	<b>-</b>	<b>38</b>	<b>0</b>	<b>-</b>	<b>3</b>	<b>-</b>	<b>-</b>	<b>38</b>	<b>49</b>	<b>21</b>	<b>2</b>	<b>0</b>	<b>3</b>
m-Endo Agar LES	16	16	0	-	0	-	-	15	0	-	1	-	-	12	62	25	2	0	2
Chromocult C Agar	26	26	0	-	0	-	-	22	0	-	2	-	-	25	44	15	0	0	1
Other/Unknown	1	1	-	-	0	-	-	1	-	-	0	-	-	1	-	-	0	0	0

# Compare the table above – the total number of results for 35/36/37 °C may differ somewhat due to different method information for coliform bacteria and *E. coli*.



### Sample A

- A strain of the thermotolerant coliform bacterium, *K. pneumoniae*, was present. Its colonies have a characteristic metallic sheen on LES and are blue on m-FC. Both media are based on lactose fermentation. The colonies are typical pink on the chromogenic enzyme-based medium CCA. However, the strain of *K. pneumoniae* is indole-negative and has no  $\beta$ -glucuronidase activity, and therefore cannot be mistaken for *E. coli* after confirmation from LES and m-FC.
- One false positive result was reported.

### Sample B

- No *E. coli* was included but two other coliform bacteria, of which one was a strain of *K. oxytoca*. That strain is able to grow in broth at 44 °C and is producing indole. It is possible to get a false positive result when colonies are selected from plates incubated at 35/36/37 °C and the indole test alone is used as criterion for *E. coli*.
- Three false positive results were reported.

### Sample C

- One characteristic *E. coli* strain was included together with another coliform bacterium, *E. cloacae*. The *E. coli* strain is positive for  $\beta$ -glucuronidase activity, indole production and gas production. It forms typical colonies on the primary growth media, metallic sheen on LES and blue on m-FC and CCA. *E. cloacae* is indole-negative and has no  $\beta$ -glucuronidase activity, meaning it cannot be mistaken for *E. coli* after confirmation.
- The distribution of the results was fairly good with a small to medium dispersion. Three false negative results and four high outliers were present.

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

The rapid method used for both these parameters was exclusively Colilert<sup>®</sup> Quanti-Tray<sup>®</sup> from the manufacturer IDEXX Inc. with incubation at 35, 36 or 37 °C. Of the 48 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 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 results from different temperatures and maximum incubation time the differences are small and inconsistent. No differences based on these criteria are therefore given.

In this PT round there were more deviant results from laboratories using 51 wells compared to 97 wells. There is also a tendency for somewhat lower average results for coliform bacteria when using 51 wells, at least in sample A. However, the number of results from 51 wells is relatively low, which makes the comparison uncertain.

There is no indication of interpretation difficulties in any sample.

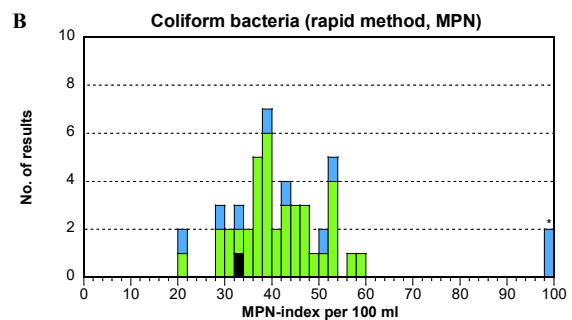
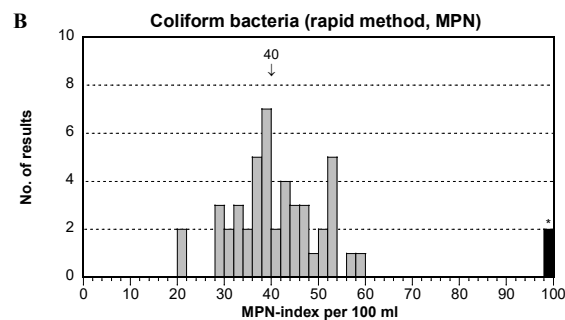
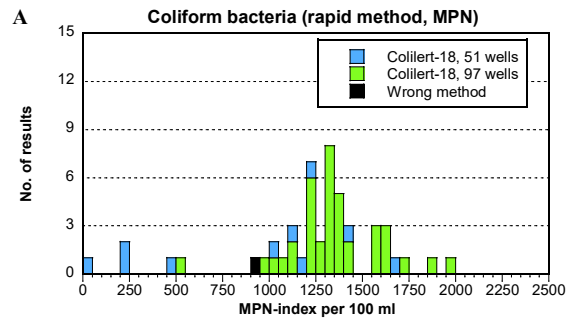
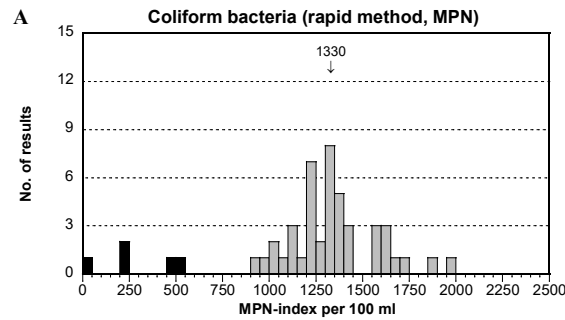
*Coliform bacteria, Rapid method with MPN*

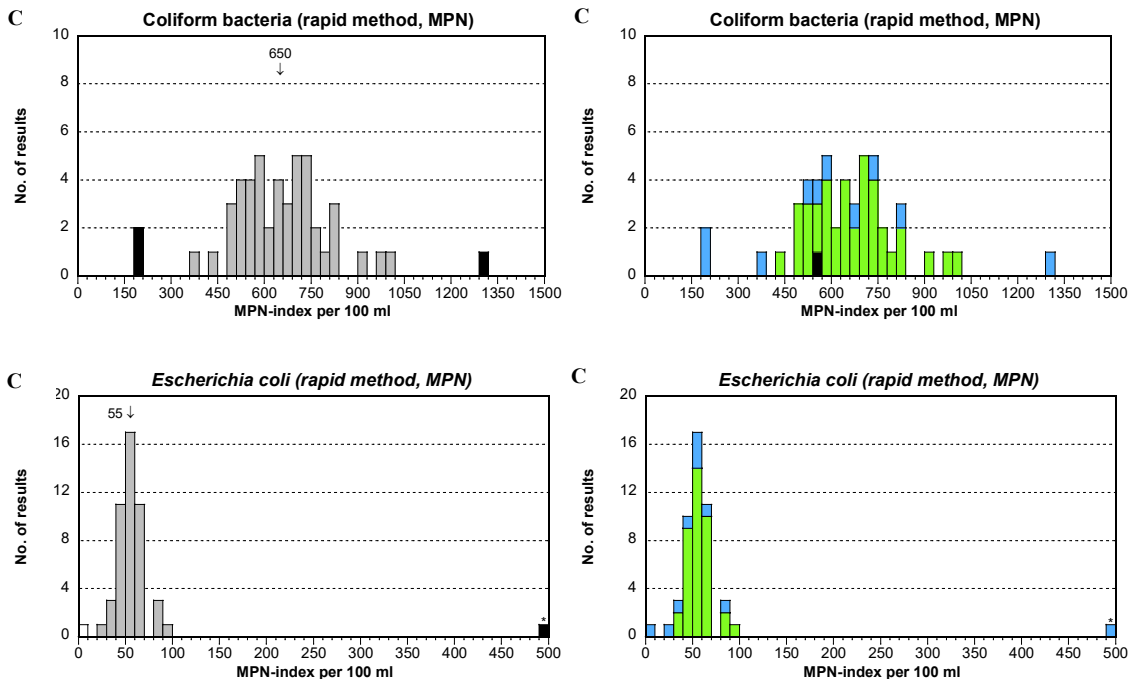
Principle	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, Rapid meth.</b>	48	43	1341	8	0	5	0	45	40	11	0	0	2	45	652	10	0	2	1
Colilert-18, 51 wells	10	6	1250	9	0	4	0	7	37	16	0	0	2	7	604	12	0	2	1
Colilert-18, 97 wells	38	37	1356	8	0	1	0	38	40	11	0	0	0	38	661	10	0	0	0
<b>Wrong method<sup>#</sup></b>	1	1	-	-	0	0	0	1	-	-	0	0	0	1	-	-	0	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>	48	48	0	-	0	-	-	46	0	-	1	-	-	46	55	12	1	0	1
Colilert-18, 51 wells	10	10	0	-	0	-	-	8	0	-	1	-	-	8	52	18	1	0	1
Colilert-18, 97 wells	38	38	0	-	0	-	-	38	0	-	0	-	-	38	55	10	0	0	0
<b>Wrong method<sup>#</sup></b>	1	1	-	-	0	-	-	1	-	-	0	-	-	1	-	-	0	0	0

# No rapid kit method but a multiple tube method based on lactose fermentation,





### Sample A

- The strain of *K. pneumoniae* is the only coliform bacterium capable of growing in the medium. It has the enzyme  $\beta$ -galactosidase and is detected as a coliform bacterium 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 *K. pneumoniae* lacks the enzyme  $\beta$ -glucuronidase and is therefore not detected as *E. coli*.
- The distribution of the coliform results was good with very small dispersion. Five low outliers were present, of which four used 51 wells.
- The distribution of the results for coliform bacteria is not as dispersed as for the MF-method (lesser CV) and the average is about 20 % higher (compare p. 6).

### Sample B

- Two different coliform bacteria, *C. freundii* and *K. oxytoca*, were included, but no *E. coli*.
- The distribution of the results was good and the dispersion was small. Two high outliers were reported for coliform bacteria. A false positive result was present for *E. coli*; this result is from a laboratory that likely mixed up their samples and thereby also reported a false negative in sample C.

### Sample C

- The strains of *E. coli* and *E. cloacae* grow in the medium and possess the enzyme  $\beta$ -galactosidase; they are therefore detected as coliform bacteria. The *E. coli* strain also has the enzyme  $\beta$ -glucuronidase and it is hence detected as *E. coli* as well.
- The average result for coliform bacteria with rapid methods was about 15 % higher than the average result for MF methods (compare p. 6).

- Two low and one high outlier were reported for coliform bacteria. One false negative result and one high outlier were reported for *E. coli*.

## **Presumptive and confirmed *Clostridium perfringens* (MF)**

The parameter to be analysed for *Clostridium perfringens* is the sum of spores and vegetative cells of *C. perfringens*. In Sweden presumptive *C. perfringens* are accepted, which is why that parameter is presented separately.

In an annex [6] to the European Drinking Water Directive from 1998 [4] it is stated that the standard EN ISO 14189:2016 with its national editions must be used after October 2017. The identical version ISO 14189 from 2013 can alternatively be used. Isolated colonies of presumptive *C. perfringens* on TSC agar are tested for acid phosphatase activity as confirmation for *C. perfringens*.

Before 2017 there was no international standard stated as reference method in the EU Directive [4]. A specific method was instead explicitly included into an annex of the directive, the use of m-CP Agar incubated at 44 °C. The method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*. A draft standard was soon accepted as an alternative, ISO/CD 6461-2:2002-12-20 based on TSC, until a finished standard was available.

The majority of participants in this PT, 37 of 46 laboratories, used the standard (EN) ISO 14189. *C. perfringens* was only present in sample B and no particular difference can there be seen between the standards. *C. bifermentans* was present as presumptive *C. perfringens* in sample C.

### *Presumptive Clostridium perfringens MF*

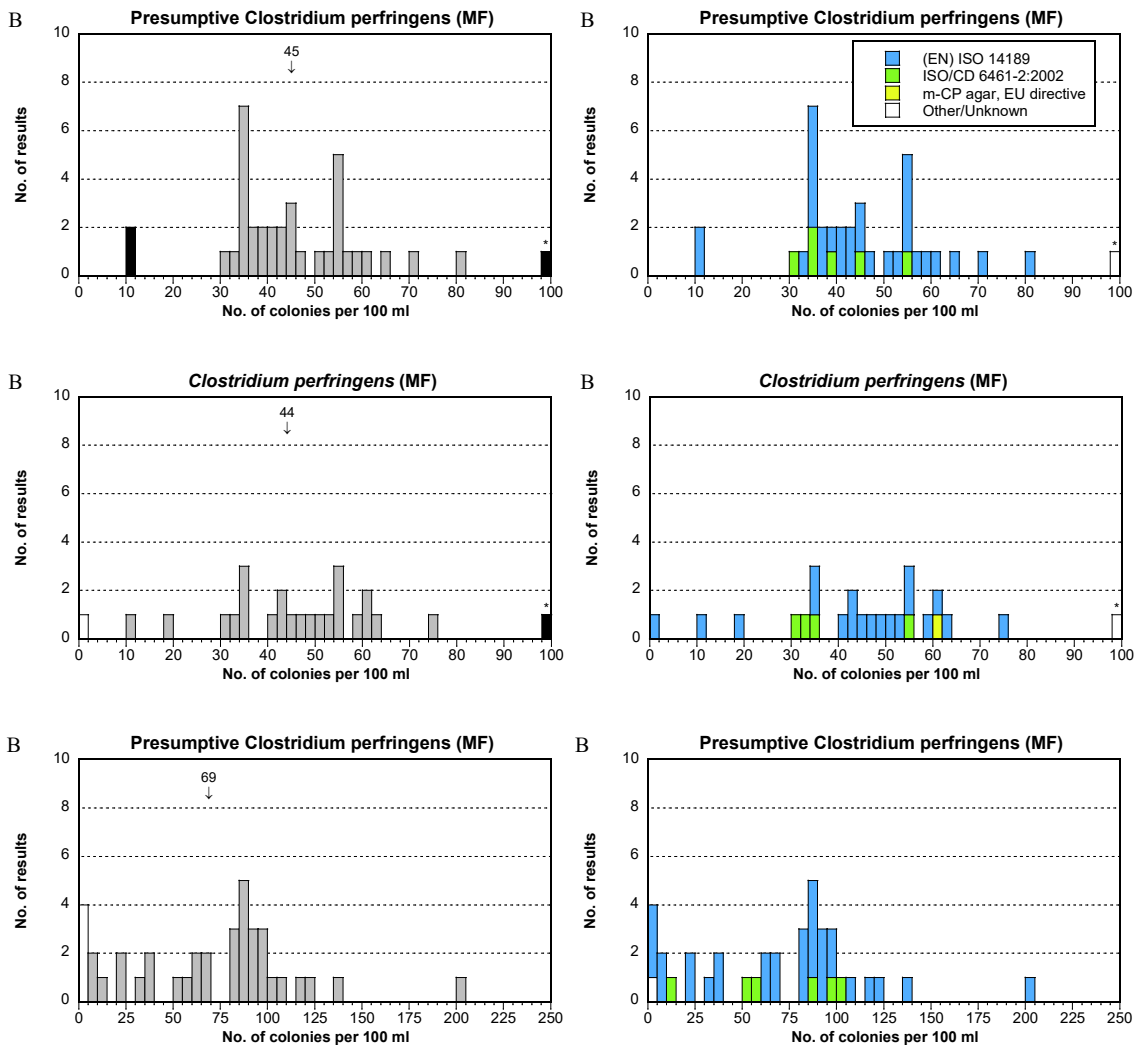
Standard/Method	N#	A					B					C						
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >		
<b>Total</b>	46	36	<b>0</b>	-	2	-	34	<b>45</b>	13	0	2	1	34	<b>69</b>	31	4	0	0
(EN) ISO 14189	37	30	<b>0</b>	-	1	-	28	<b>47</b>	13	0	2	0	28	<b>71</b>	31	3	0	0
ISO/CD 6461-2:2002	7	6	<b>0</b>	-	0	-	6	<b>39</b>	11	0	0	0	6	<b>63</b>	30	0	0	0
m-CP agar, EU-direct.	1	0	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Other/Unknown	1	0	-	-	1	-	0	-	-	0	0	1	0	-	-	1	0	0

### *Clostridium perfringens MF*

Standard/Method	N#	A					B					C						
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >		
<b>Total</b>	46	24	<b>0</b>	-	2	-	23	<b>44</b>	18	1	0	1	25	<b>0</b>	-	5	-	-
(EN) ISO 14189	37	19	<b>0</b>	-	1	-	18	<b>44</b>	19	1	0	0	20	<b>0</b>	-	3	-	-
ISO/CD 6461-2:2002	7	4*	<b>0</b>	-	0	-	4*	<b>38</b>	-	0	0	0	3*	<b>0</b>	-	2	-	-
m-CP agar, EU-direct..	1	1*	<b>0</b>	-	0	-	1*	<b>60</b>	-	0	0	0	1*	<b>0</b>	-	0	-	-
Other/Unknown	1	0	-	-	1	-	0	-	-	0	0	1	1*	<b>0</b>	-	0	-	-

# The sum of laboratories that have reported results for presumptive *C. perfringens*, and/or *C. perfringens*

\* Mean value is given for comparison despite few results



### Sample A

- No presumptive *C. perfringens* was included. Despite this, two false positive results were present for both presumptive and confirmed *C. perfringens*.

### Sample B

- A strain of *C. perfringens* was included. The colour of the colonies on TSC can vary from pale grey-brown to completely black, depending on the condition and reduction potential of the medium.
- Two low and one high outlier were present in the presumptive test and one high outlier and a false negative result were present for *C. perfringens*.
- The distributions of the results were fairly good and dispersions small for both presumptive and confirmed *C. perfringens*.

### Sample C

- A strain of *C. bifermentans* was included. The strain grows on TSC with small, black to almost transparent presumptive colonies. Confirmation shows that the colonies are not *C. perfringens*.



- The dispersion (CV) was large. Four false negative results were present for presumptive *C. perfringens*.
- Five false positive results were present in the analyses of *C. perfringens*. Either no confirmation was made or the confirmation was misinterpreted.

## **Moulds and yeasts (MF)**

---

Of the 38 laboratories that analysed moulds and yeasts, 27 reported that they followed the Swedish standard SS 028192. This standard is also partly used in Finland under their own national designation SFS 5507. Sometimes it is modified regarding media composition, for example dichloran (DRBC) may be used.

Various names are reported for the media linked to the use of SS 028192 and SFS 5507. These are: Cooke Rose Bengal Agar base, Rose Bengal Agar base, Rose Bengal Agar, Rose Bengal Chloramphenicol Agar and Dichloran Rose Bengal Chloramphenicol Agar (DRBC). According to the original standard, dichloran should not be included in the medium (and thus DRBC should not be used) but instead Rose Bengal and the two stronger inhibitory substances chlortetracycline and chloramphenicol.

Here, the medium stated by the laboratories is shown, and a separation is made for those that used any form of "Rose Bengal Agar" (RBC), DRBC, ME and OGYE. Five laboratories from various countries stating DRBC in conjunction with SS 028192, SFS 5507 or "Standard methods" [5], which comprise the group DRBC "Water" in the tables. Three Norwegian laboratories instead followed NMKL 98:2005, modified to be used with DRBC. These comprise the group DRBC "Food" in the tables. Malt Extract Agar was used by four Finnish laboratories—of which one laboratory stated the use of NMKL 98:2005—and by one laboratory from Tanzania. These five laboratories comprise the group ME. One Finnish laboratory using Oxytetracycline Glucose Extract Agar (OGYE) based on other methods/standards. Several of the different groups contain so few results (<5) that discussion of possible differences is not meaningful. The mean values are however still given for comparison.

The laboratories that did not use any magnification tend to have more deviant results for both moulds and yeasts.

### **Sample A**

- The mould *Acremonium strictum* and the yeast *Hanseniaspora uvarum* were included in similar concentrations. With the exception of the many zero results for moulds and some other deviating results, the result distributions were relatively good. The relative dispersions (CV) of the accepted results were very small for both moulds and yeasts.
- There were 13 false negative results for moulds and three for yeasts, one low and three high outliers for moulds and two low outliers for yeasts.
- The false negative results for moulds are probably caused by the presence of small, undeveloped *A. strictum* colonies. After seven days of incubation, they often have a very pale mycelium without mature spores (colourless colonies). It is likely that

these laboratories did not see these colonies at all, or they did not interpret them as mould colonies.

### Sample B

- Neither moulds nor yeasts were included. Despite this, four false positive results were reported for moulds and one false positive result for yeasts.

### Sample C

- No yeasts were included in the sample but the mould *Cladosporium cladosporioides*.
- Five false negative results and one high outlier were present for moulds, and two false positive results for yeasts. All false negative and false positive results were reported by laboratories that used media other than RBC. All laboratories with deviating results also reported at least one more deviating result, which implies those particular laboratories had more general problems.
- The distribution of results of the mould was fairly good with medium dispersion.

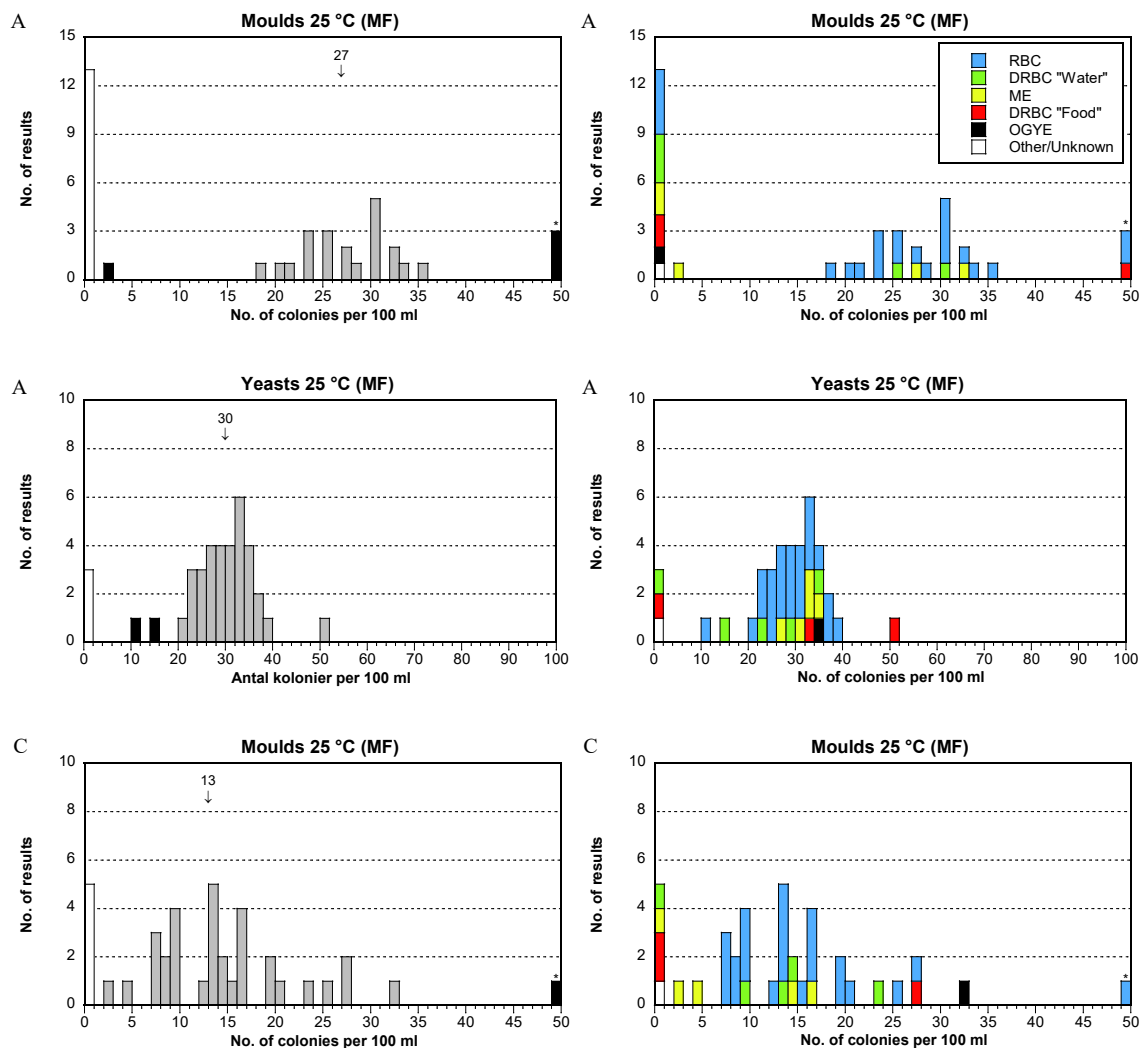
#### Moulds MF

Standard, Method	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	38	21	27	9	13	1	3	33	0	-	4	-	-	32	13	26	5	0	1
RBC	23	17	26	9	4	0	2	21	0	-	1	-	-	22	13	21	0	0	1
DRBC "Water"	5	2*	27	-	3	0	0	4*	0	-	1	-	-	4*	14	-	1	0	0
ME	5	2*	29	-	2	1	0	3*	0	-	2	-	-	4*	8	-	1	0	0
DRBC "Food"	3	0	-	-	2	0	1	3*	0	-	0	-	-	1*	27	-	2	0	0
OGYE	1	0	-	-	1	0	0	1*	0	-	0	-	-	1*	32	-	0	0	0
Other/Unknown	1	0	-	-	1	0	0	1*	0	-	0	-	-	0	-	-	1	0	0
<b>Magnification</b>																			
None	19	9	28	6	7	1	2	16	0	-	3	-	-	15	14	23	3	0	1
1,1-4,9x	5	3*	27	-	1	0	1	4*	0	-	1	-	-	3*	13	-	2	0	0
5-11,9x	13	8	26	11	5	0	0	12	0	-	0	-	-	13	13	28	0	0	0
16-19,9x	1	1*	20	-	0	0	0	1*	0	-	0	-	-	1*	7	-	0	0	0

#### Yeasts MF

Standard, Method	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total</b>	38	33	30	9	3	2	0	36	0	-	1	-	-	36	0	-	2	-	-
RBC	23	22	29	8	0	1	0	22	0	-	0	-	-	23	0	-	0	-	-
DRBC "Water"	5	3*	28	-	1	1	0	5	0	-	0	-	-	4*	0	-	1	-	-
ME	5	5	31	5	0	0	0	4*	0	-	1	-	-	4*	0	-	1	-	-
DRBC "Food"	3	2*	41	-	1	0	0	3*	0	-	0	-	-	3*	0	-	0	-	-
OGYE	1	1*	34	-	0	0	0	1*	0	-	0	-	-	1*	0	-	0	-	-
Other/Unknown	1	0	-	-	1	0	0	1*	0	-	0	-	-	1*	0	-	0	-	-
<b>Magnification</b>																			
Ingen	19	16	31	6	2	1	0	18	0	-	1	-	-	18	0	-	1	-	-
1,1-4,9x	5	4*	31	-	1	0	0	5	0	-	0	-	-	4*	0	-	1	-	-
5-11,9x	13	12	29	6	0	1	0	12	0	-	0	-	-	13	0	-	0	-	-
16-19,9x	1	1*	20	-	0	0	0	1*	0	-	0	-	-	1*	0	-	0	-	-

\* Mean value is given for comparison despite few results

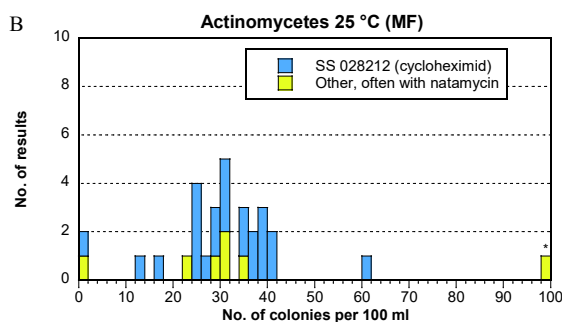
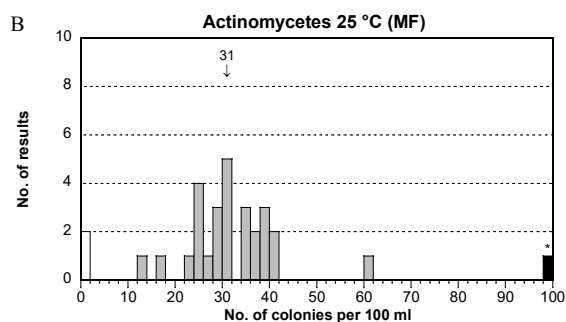


## Actinomycetes (MF)

The analysis of actinomycetes is a prescribed method for drinking water monitoring according to Swedish regulations, and therefore mainly Swedish laboratories perform this analysis. It is performed according to the Swedish standard for actinomycetes in water, SS 028212 (1994). Seven Finnish laboratories performed the analysis based on other methods, and are placed together in the group Other. Notably, they stated the use of natamycin as the selective substance instead of cycloheximide, and they detected actinomycetes after seven and 14 days. The base agar medium varied within this group, but all laboratories used other media than Actinomycete Isolation Agar (ACTA), which is the base medium in the Swedish standard.

The averages of the two groups ACTA and Other in sample B are similar. However, the dispersion (CV) was smaller within the group Other.

Medium/Standard	N	A					B					C									
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>		
<b>Total</b>	30	29	0	–	1	–	–	27	31	14	2	0	1	–	–	30	0	–	0	–	–
ACTA (SS 028212)	23	23	0	–	0	–	–	22	31	15	1	0	0	–	–	23	0	–	0	–	–
Other	7	6	0	–	1	–	–	5	29	7	1	0	1	–	–	7	0	–	0	–	–



### Sample A

- No actinomycetes were included in the sample. One false positive result was present.

### Sample B

- One actinomycete within the group *Streptomyces* sp. was included. The distribution of the results was good and the average dispersion small.
- Two false negative results and one high outlier were present.

### Sample C

- The sample contained no actinomycetes and there were no false positive results.

## Culturable microorganisms 22 °C, 3 days

Sixty-eight of the 69 laboratories followed EN ISO 6222:1999, which prescribes the use of Yeast extract Agar (YEA). However, seven of the laboratories that followed EN ISO 6222:1999 instead used Plate Count Agar. One laboratory used YEA in conjunction with Standard methods [5]. The majority of the laboratories stated that they count both bacterial and fungal colonies. Ten laboratories stated that they do not count fungi and two more that they count yeasts but not moulds.

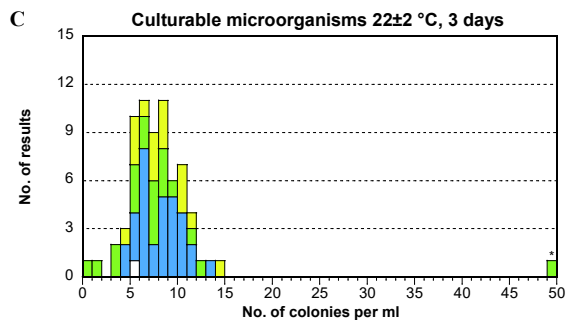
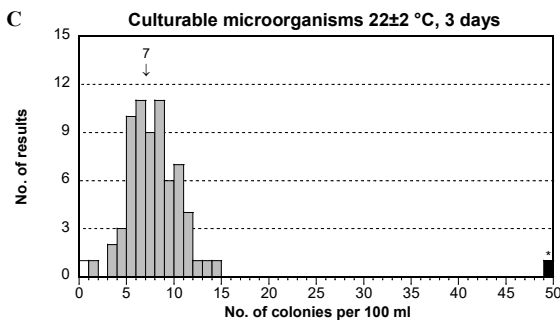
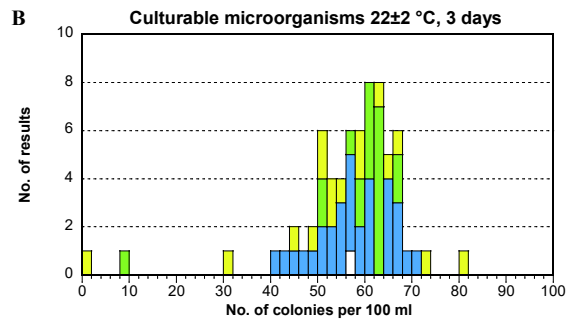
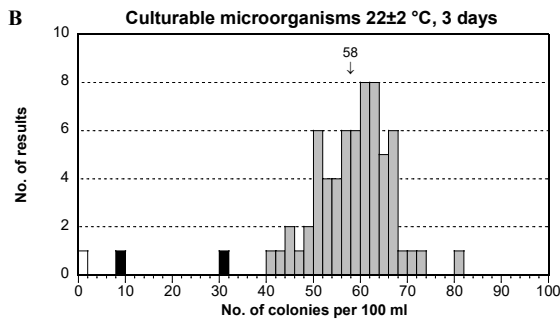
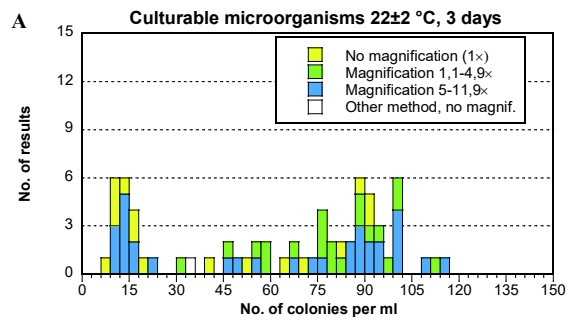
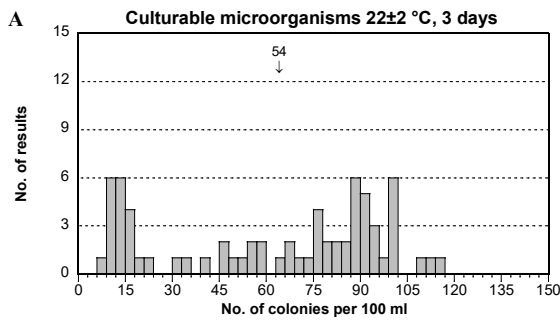
Since all except one laboratory refer to EN ISO 6222:1999, differences among method variants are relevant to discuss only for these. Results are shown for culture media and the magnification at reading.

As usual, it is difficult to find any consistent method differences. In sample A, Plate Count Agar gave, as sometimes before, lower result than YEA. This probably depends on the organisms that are present in the samples. Also in sample A, small colonies of *Sphingomonas* sp. may have appeared already after three days of incubation, even though they are usually only visible when using magnification on the fourth day of incubation. If *Sphingomonas* sp. appeared already after three days, it is apparent that

the magnification that was used had an impact, since the results are lower when no magnification was used.

For samples B and C there are no apparent differences.

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>69</b>	<b>69</b>	<b>54</b>	<b>35</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>67</b>	<b>58</b>	<b>6</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>69</b>	<b>7</b>	<b>18</b>	<b>1</b>	<b>0</b>	<b>1</b>
<i>EN ISO 6222</i>	68	68	55	35	0	0	0	63	58	7	1	2	0	66	7	18	1	0	1
<i>Medium</i>																			
Yeast extract Agar	61	61	58	34	0	0	0	58	59	7	1	0	0	60	7	18	1	0	0
Plate Count Agar	7	7	34	46	0	0	0	5	54	5	0	2	0	6	8	13	0	0	1
<i>Magnification</i>																			
None	16	16	36	45	0	0	0	14	58	8	1	1	0	16	8	17	0	0	0
1,1–4,9×	20	20	76	14	0	0	0	18	60	4	0	1	0	18	6	24	1	0	1
5–11,9×	32	32	53	39	0	0	0	31	57	7	0	0	0	32	7	15	0	0	0
<i>Other method</i>	1	1	–	–	0	0	0	1	–	–	0	0	0	1	–	–	0	0	0



### Sample A

- The colonies mainly consisted of *K. pneumoniae*, but some laboratories have probably also detected *Sphingomonas* sp. *Staphylococcus warneri*, may also have contributed with occasional colonies.
- The distribution of the results was not good but dispersed with a tendency towards two peaks. Therefore, no outliers could be identified. The common average is in the middle between the two peaks and is therefore misleading.
- The first peak (6-22 cfu/ml) represents results where only *K. pneumoniae* have been counted. This is illustrated by comparison with the average result for coliform bacteria with the rapid method (13 cfu/ml). The second peak (31-115 cfu/ml) consists of *K. pneumoniae* and a varying number of *Sphingomonas* sp. Since the colonies of the latter are very small after only three days of incubation, usually not visible until the fourth day, the differences in outcomes are likely caused by the differences in how many *Sphingomonas* sp. colonies that were counted. The colonies are probably counted after somewhat varying incubation times, and consequently varying number of visible colonies can be expected. The visibility of colonies is of course also indirectly affected by the magnification that is used.

### Sample B

- The colonies mainly consisted of the strain of *S. saprophyticus* but individual colonies of the coliform bacteria may also have appeared.
- The distribution of the results was good with very small dispersion. One false negative and two low outliers were present.

### Sample C

- Only a few colonies of culturable microorganisms appeared at 22 °C. The majority consisted of *Enterobacter cloacae*. One false negative result and one high outlier were obtained.

## Slow-growing bacteria 22 °C, 7 days

---

Thirty-nine laboratories performed the analysis of slow-growing bacteria. The parameter is mandatory to monitor according to the Swedish Drinking water ordinances, and therefore a custom method is adopted and used by the Swedish laboratories. Today, a modified version of this method is used in the standard EN ISO 6222:1999, which prescribes incubation on yeast extract agar (YEA). The modification includes: incubation at 22±1 °C for seven days, using at least 4× (preferentially 10×) magnification when reading the plates, and that only bacterial colonies shall be counted.

There is an ongoing effort within ISO to develop a standard method for the parameter "slow-growing microorganisms". The current proposal is to use a more nutrient depleted medium than YEA, namely "Reasoner's 2 Agar" (R2A). In this PT, R2A was used by six laboratories that constitute a separate group in the table and the histograms.

Twenty-five laboratories stated they do not include fungal colonies when present, while 10 stated that they include both moulds and yeasts. Another four laboratories stated that they only include yeasts.

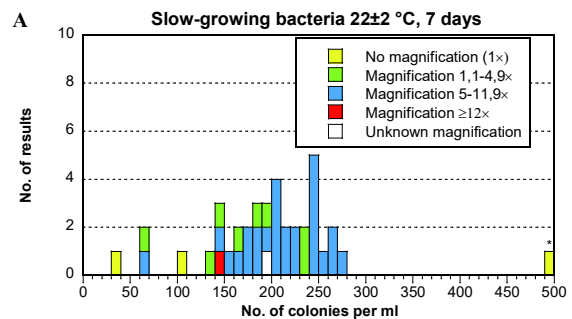
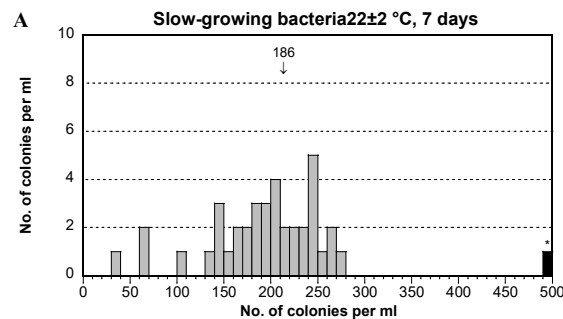
For sample A, the few laboratories that used R2A on average reported lower results than those that used YEA. However, these differences are probably not due to the medium used, but rather the magnification that was used when counting the colonies. This is apparent since the differences are even more pronounced when comparing different degrees of magnification.

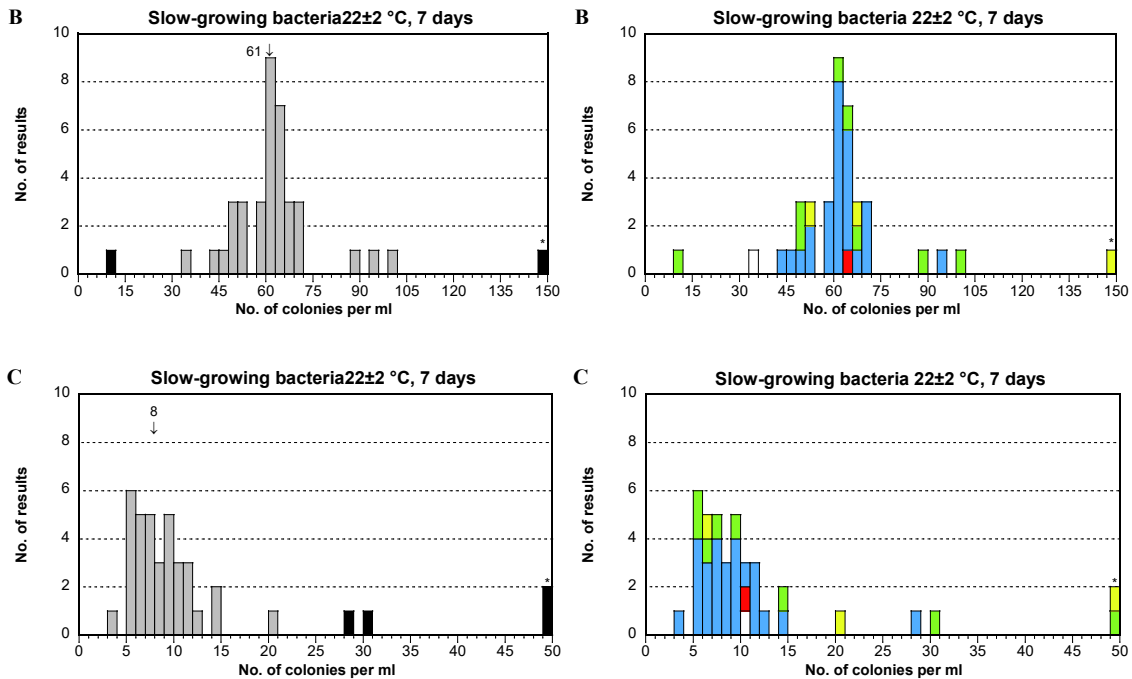
In both samples B and C there are no apparent differences in recovery between culturable microorganisms and slow-growing bacteria. However for sample A, the average number of slow-growing bacteria is more than triple that of culturable microorganisms. Only this sample contained a slow-growing bacterium and the outcome illustrates the problem with equal evaluation of them. These colonies are often small and therefore difficult to count without magnification, which probably explains the difference in recovery between the laboratories for sample A.

Most Swedish laboratories normally use YEA and 10 × magnification. This is probably the explanation for the fairly equal and high average results for YEA and the magnification 5–11 ×.

Group of result	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
<b>Total, all results</b>	<b>39</b>	<b>38</b>	<b>186</b>	<b>18</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>37</b>	<b>61</b>	<b>10</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>35</b>	<b>8</b>	<b>19</b>	<b>0</b>	<b>0</b>	<b>4</b>
<i>Medium</i>																			
Yeast extract Agar	31	30	<b>197</b>	17	0	0	1	30	<b>61</b>	7	0	0	1	29	<b>8</b>	17	0	0	2
"Reasoner's 2 Agar"	6	6	<b>148</b>	19	0	0	0	5	<b>60</b>	19	0	1	0	4*	<b>10</b>	–	0	0	2
Other/Unknown	2	2*	<b>145</b>	–	0	0	0	2*	<b>69</b>	–	0	0	0	2*	<b>12</b>	–	0	0	0
<i>Magnification</i>																			
None	3	2*	<b>69</b>	–	0	0	1	2*	<b>60</b>	–	0	0	1	2*	<b>12</b>	–	0	0	1
1,1–4,9×	8	8	<b>164</b>	19	0	0	0	7	<b>68</b>	13	0	1	0	6	<b>7</b>	21	0	0	2
5–11,9×	26	26	<b>206</b>	13	0	0	0	26	<b>61</b>	7	0	0	0	25	<b>8</b>	17	0	0	1
≥ 12×	1	1*	<b>143</b>	–	0	0	0	1*	<b>63</b>	–	0	0	0	1*	<b>10</b>	–	0	0	0
Unknown	1	1*	<b>190</b>	–	0	0	0	1*	<b>35</b>	–	0	0	0	1*	<b>10</b>	–	0	0	0

\* Mean value is given for comparison despite few results





### Sample A

- The colonies mainly consisted of *Sphingomonas* sp. but also to some degree of *K. pneumoniae* and perhaps some occasional yeast colonies.
- The distribution of the results was better than for culturable microorganisms with only one broad peak and a few lower results. The dispersion (CV) was small, in contrast to the large CV for culturable microorganisms. One high outlier was present.
- The average result was higher with the magnification 5–11.9× compared to when a lower or no magnification was used. In general, this indicates the impact the magnification has for proper quantification of the strain of slow-growing bacterium included here. It is most plausible that this also applies to many other typical slow-growing bacteria that form small colonies.

### Sample B

- The result distribution for the culturable microorganisms present in the sample was good. The approximately 60 colonies mainly consisted of the strain of *S. saprophyticus*, which could be counted as culturable microorganisms already after three days. No specific slow-growing bacterium was present in the sample.
- One high and one low outlier were present.

### Sample C

- Only a few colonies appeared at 22 °C after seven days. The majority consisted of *Enterobacter cloacae* which could be counted as a culturable microorganism already after three days. No colonies of a specific slow-growing bacterium appeared due to too low concentration. Four 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 summarising 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 together with their z-scores.

*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**

Fifteen laboratories have more than one deviating result. Laboratory 4339 seem to have mixed up whole samples and the corresponding sample numbers are crossed out in annex A. A few laboratories may have performed individual incorrect calculations from their colony readings to the final concentrations. Three laboratories have reported unreasonably high results for specific analyses, which may indicate misunderstandings regarding the analysis or reporting of method data, use of incorrect method or contamination of the samples in their own laboratory.

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

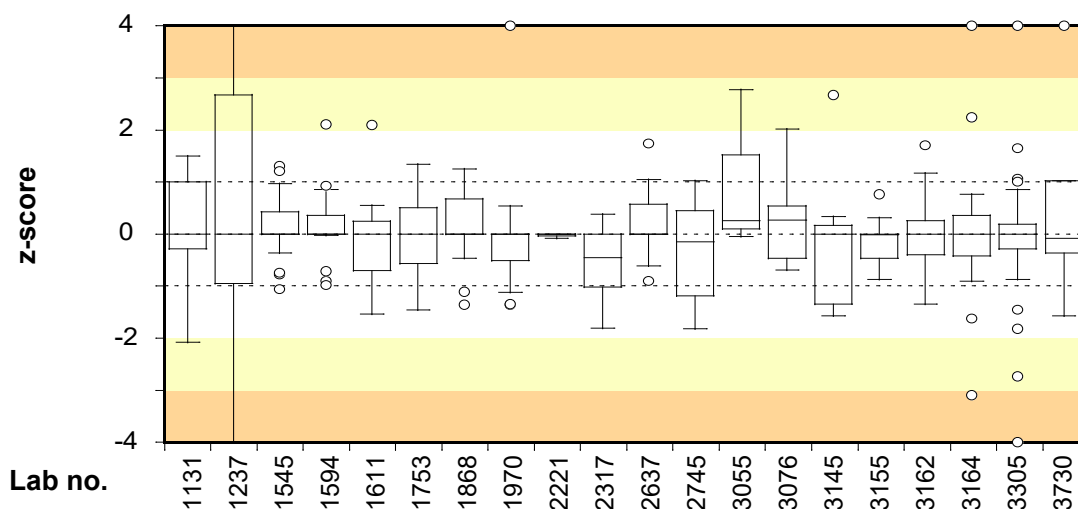
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are reported in annex B but are not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control 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.

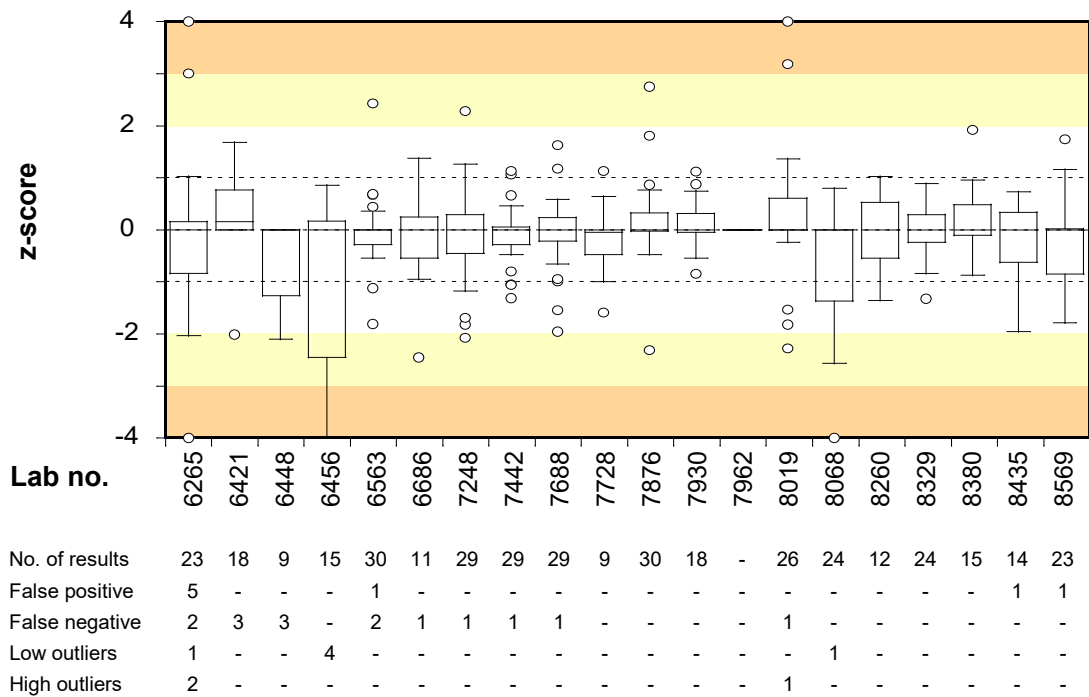
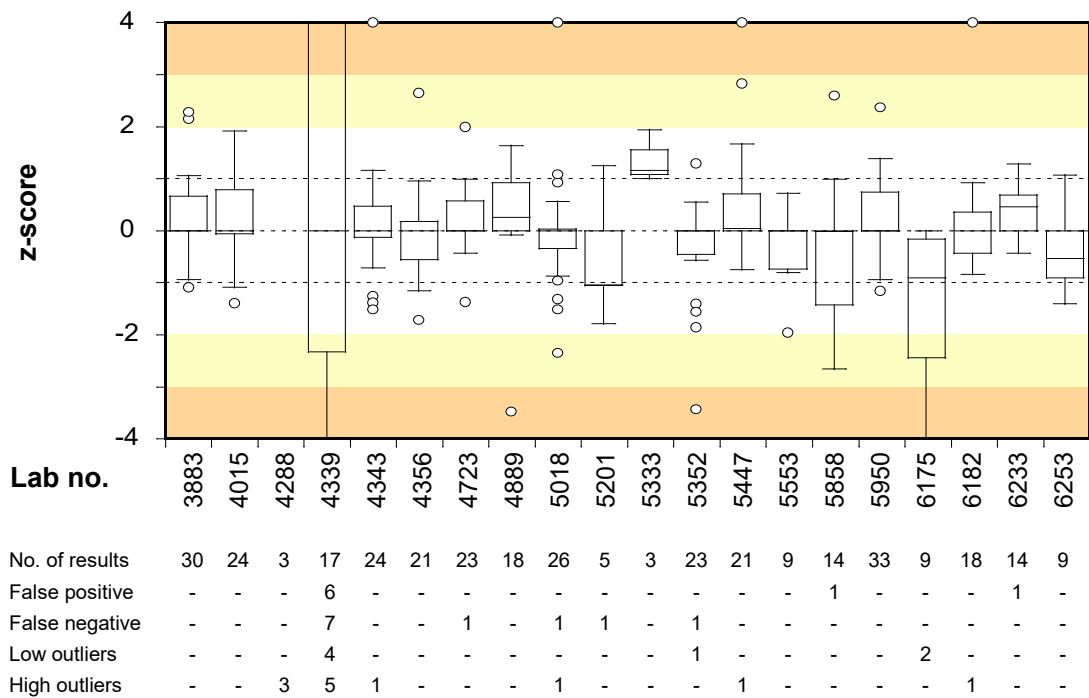
### Box plots and numbers of deviating results for each participating laboratory

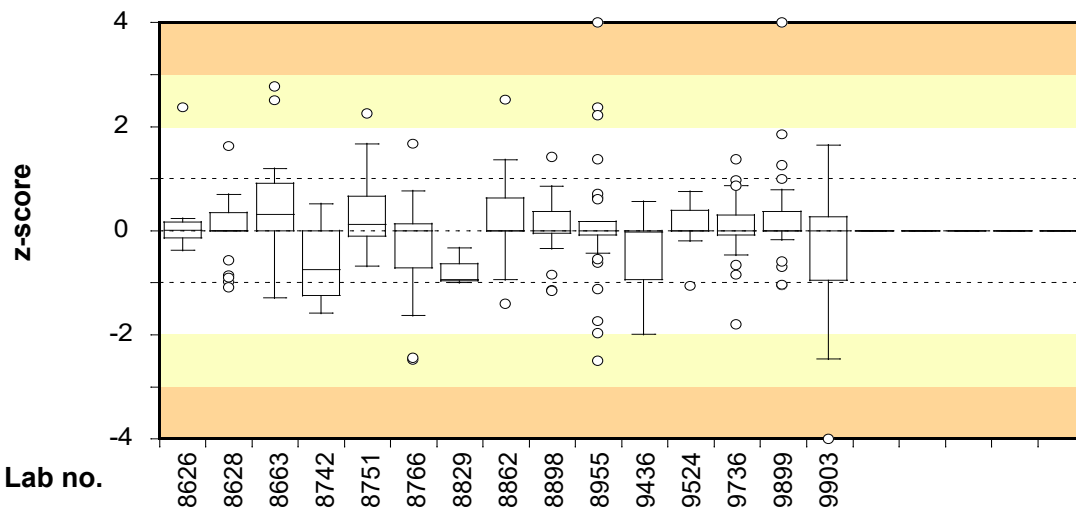
- z-scores are calculated from the formula  $z = (x - mv) / s$  (see annex A).
- A correct result "zero" will get  $z = 0$  when there is no target organism present.
- False results do not generate z-scores and are not included in 'No. of results'.
- The outliers are included in the plots after recalculation 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  $> [\text{largest value of the box} + 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$



No. of results	15	15	31	23	18	24	29	21	3	9	15	8	3	6	8	12	24	20	27	6
False positive	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	1	-
False negative	-	-	1	1	-	-	1	-	-	-	-	-	-	-	1	-	-	-	2	-
Low outliers	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
High outliers	-	4	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	2	1	1





Lab no.

No. of results	8	23	20	9	12	30	3	33	30	29	30	21	24	30	24
False positive	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
High outliers	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-

## Test material, quality controls and processing of data

### Description of the test material

The 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>Klebsiella pneumoniae</i>	186	CCUG 45102	1500
	<i>Acremonium strictum</i>	502	CBS, verified	31
	<i>Hanseniaspora uvarum</i>	555	CF SQE 77 #	35
	<i>Sphingomonas</i> sp.	547	CCUG 36955	170 *
	<i>Staphylococcus warneri</i>	189	CCUG 45143	<1 *
B	<i>Citrobacter freundii</i>	091	CCUG 43597	26
	<i>Klebsiella oxytoca</i>	553	From water	28
	<i>Clostridium perfringens</i>	442	CCUG 43593	43
	<i>Streptomyces</i> sp.	548	From water	35
	<i>Staphylococcus saprophyticus</i>	013	CCUG 45100	62 *
C	<i>Escherichia coli</i>	082	CCUG 45097	44
	<i>Enterobacter cloacae</i>	451	CCUG 30205	600
	<i>Clostridium bifermentans</i>	009	CCUG 43592	70
	<i>Cladosporium cladosporioides</i>	488	CBS, verified	19
	<i>Sphingomonas</i> sp.	547	CCUG 36955	<1 *

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; CBS: Centraalbureau voor Schimmelcultures, Utrecht, Holland; – or "From water" indicate a strain from our own "culture collection that has not yet been typed at another culture collection

# Designation of an older 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 % of the number of vials produced from the sample mixtures. The largest differences between vials were 4, 6 and 7 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; shaded rows are not used for performance assessing

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>	15 <sup>a</sup>	0.6	1.5	54	0.9	1.3	59 <sup>b</sup>	0.5	1.2
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	13 <sup>a</sup>	0.9	1.	–	–	–	3 <sup>b</sup>	1.6	7.8
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	–	–	–	–	–	–	4 <sup>b</sup>	1.2	3.4
Presumptive <i>Clostridium perfringens</i> (MF) <i>TSC Agar according to SS-EN ISO 14189:2016</i>	–	–	–	21 <sup>c</sup>	0.7	1.4	7 <sup>b</sup>	0.9	2.1
Moulds (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	31	0.6	1.3	–	–	–	19	1.6	1.8
Yeasts (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	35	1.7	1.6	–	–	–	–	–	–
Actinomycetes (MF) <i>Actinomycete Isolation Agar with cycloheximide according to SS 028212</i>	–	–	–	18 <sup>c</sup>	1.2	1.6	–	–	–
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	16	0.8	1.6	62	2.6	1.5	10	0.8	1.7
Slow-growing bacteria, 7d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999 modified</i>	186	0.9	1.1	–	–	–	11	1.3	1.9

1 5 vials for sample A and B and 10 vials for sample C analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, analysed 16, 14 and 15 weeks prior the testing round for the sample A, B and C, respectively

a Determined for the volume 1 ml

b Determined for the volume 10 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 volumes that were 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 could 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,  $\log_{10}$  transformation of results is seldom routine as there are usually low concentrations. 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 dispersion.

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
<b>n</b>		32	31	32	52	50	52	26	26	26	53	51	53	49	48	49	49	48	49
<b>Min</b>		600	32	290	39	25	80	0	0	12	0	0	0	32	20	200	0	0	0
<b>Max</b>		2000	4533	783	2000	4533	1150	2000	65	1030	200	190	490	1986	770	1300	0	61	520
<b>Median</b>		1200	44	573	1190	41.5	571	1053	0	44.5	0	0	50	1300	39	647.5	0	0	57
<b>Mean</b>					1117	41	554				0	0	50	1330	40	650	0	0	55
<b>CV (%)</b>					14	11	13				-	-	20	8	11	10	-	-	12
<b>False positive</b>					0	0	0				1	3	0	0	0	0	0	1	0
<b>False negative</b>					0	0	0				0	0	3	0	0	0	0	0	1
<b>Outliers, low</b>					2	0	1				0	0	0	5	0	2	0	0	0
<b>Outliers, high</b>					0	4	0				0	0	4	0	2	1	0	0	1
<b>Low limit OK</b>		600	32	290	400	25	230	0	0	12	0	0	20	920	20	384	0	0	28
<b>High limit OK</b>		2000	4533	783	2000	66	1150	2000	65	1030	0	0	120	1986	59	1000	0	0	91
<b>mv</b> ( $\sqrt{\text{Mean}}$ )					33.425	6.421	23.529				0.000	0.000	7.041	36.475	6.305	25.492	0.000	0.000	7.390
<b>s</b> ( $\text{CV} \cdot \text{mv} / 100$ )					4.776	0.676	3.151				0.000	0.000	1.409	3.020	0.716	2.585	0.000	0.000	0.859
<b><math>u_{\text{rel,mv}}</math> (%)</b> ( $100 \cdot s / \sqrt{n_{\text{mv}}}$ )					2.0	1.6	1.9						3.0	1.2	1.7	1.5			1.7
<b>x</b> ( $\sqrt{\text{Result}}$ )																			
<b>z</b> ( $(x - \text{mv}) / s$ )																			

# cfu/ml

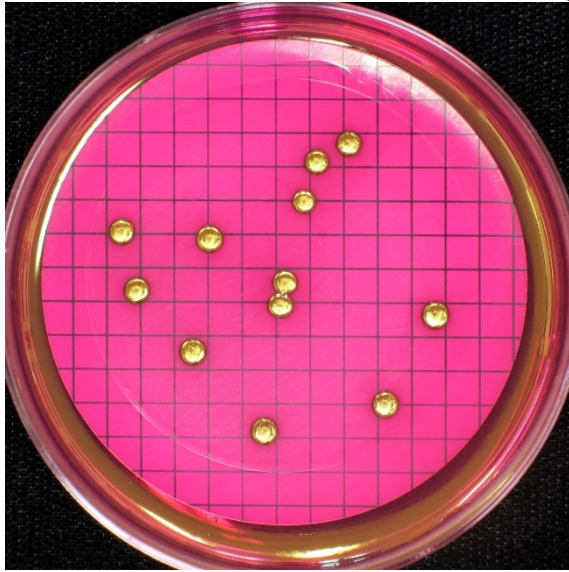
Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days <sup>#</sup>			Slow-growing bacteria 22 °C, 7 days <sup>#</sup>			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
38	37	38	26	25	26	38	37	38	38	37	38	30	30	30	69	67	69	39	39	39	n
0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	39	11	3	Min
850	470	200	850	470	100	300	43	750	50	28	300	33	260	0	115	81	81	12000	3400	6100	Max
0	43	84	0	46	0	27	0	13	30	0	0	0	31	0	73	59	7	199	61	8	Median
0	45	69	0	44	0	27	0	13	30	0	0	0	31	0	54	58	7	186	61	8	Mean
-	13	31	-	18	-	9	-	26	9	-	-	-	14	-	35	6	18	18	10	19	CV (%)
2	0	0	2	0	5	0	4	0	0	1	2	1	0	0	0	0	0	0	0	0	False pos.
0	0	4	0	1	0	13	0	5	3	0	0	0	2	0	0	1	1	0	0	0	False neg.
0	2	0	0	0	0	1	0	0	2	0	0	0	0	0	0	2	0	0	1	0	Outliers <
0	1	0	0	1	0	3	0	1	0	0	0	0	1	0	0	0	1	1	1	4	Outliers >
0	31	5	0	11	0	18	0	2	20	0	0	0	13	0	6	41	1	39	35	3	Low limit
0	80	200	0	75	0	35	0	32	50	0	0	0	60	0	115	81	14	277	100	20	High limit
0.000	6.726	8.315	0.000	6.623	0.000	5.178	0.000	3.653	5.471	0.000	0.000	0.000	5.560	0.000	7.379	7.627	2.670	13.628	7.831	2.828	mv
0.000	0.853	2.559	0.000	1.210	0.000	0.450	0.000	0.951	0.503	0.000	0.000	0.000	0.792	0.000	2.590	0.495	0.481	2.388	0.767	0.547	s
	2.2	5.3		3.8		1.9		4.6	1.6				2.7		4.2	0.8	2.2	2.8	1.6	3.3	u <sub>rel,mv</sub> (%)
																					x
																					z





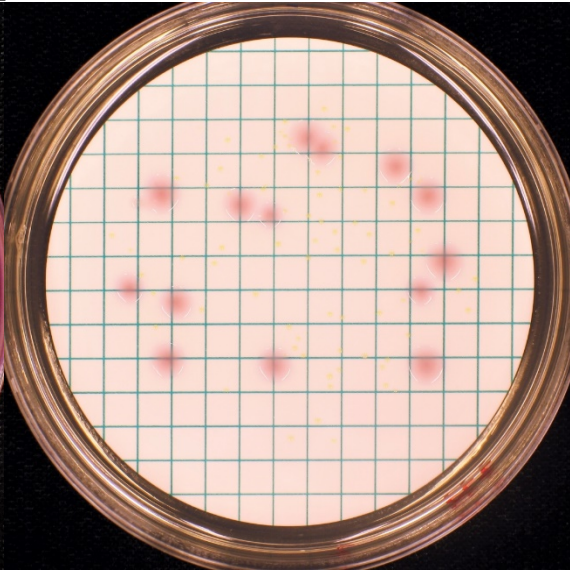
Sample A

m-Endo Agar LES, 37 °C



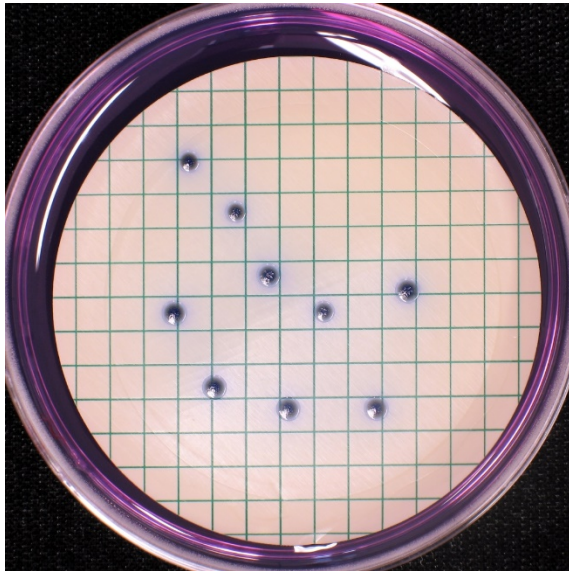
1 ml

Chromocult Coliform Agar, 37 °C



1 ml

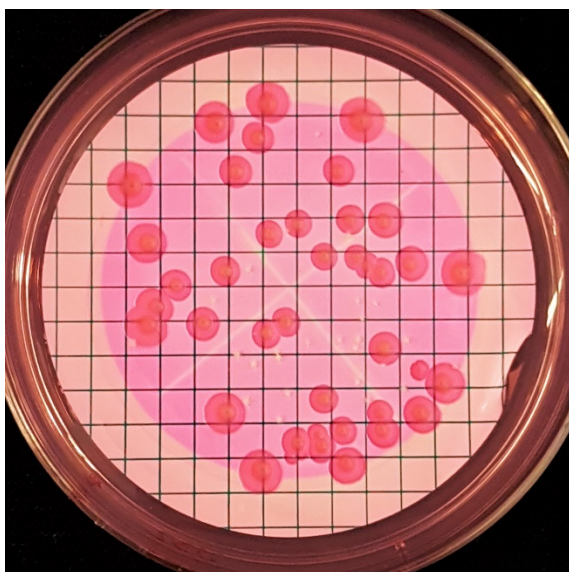
m-FC Agar, 44 °C



1 ml

m-TSC Agar, 44 °C

m-RBCC Agar, 25 °C



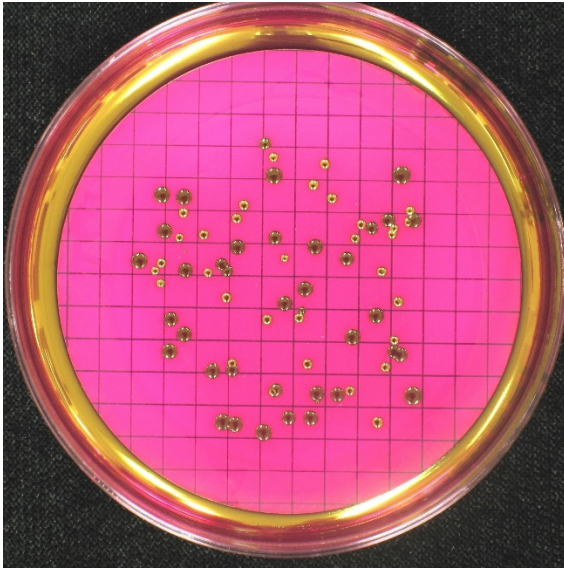
100 ml, 7 days

Actinomycete Isolation Agar, 25 °C



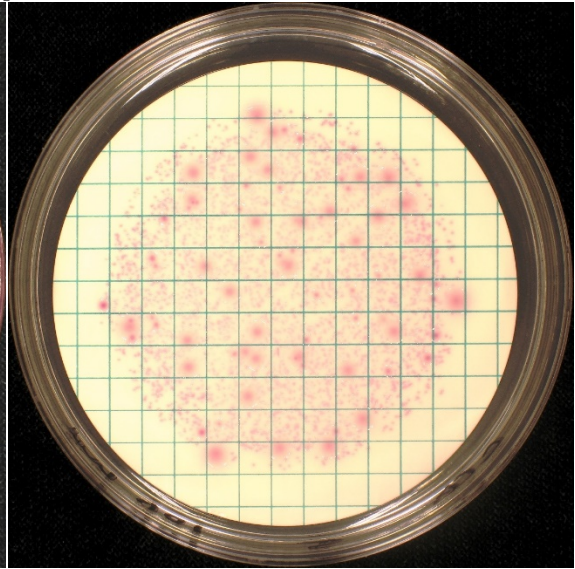
**Sample B**

**m-Endo Agar LES, 37 °C**



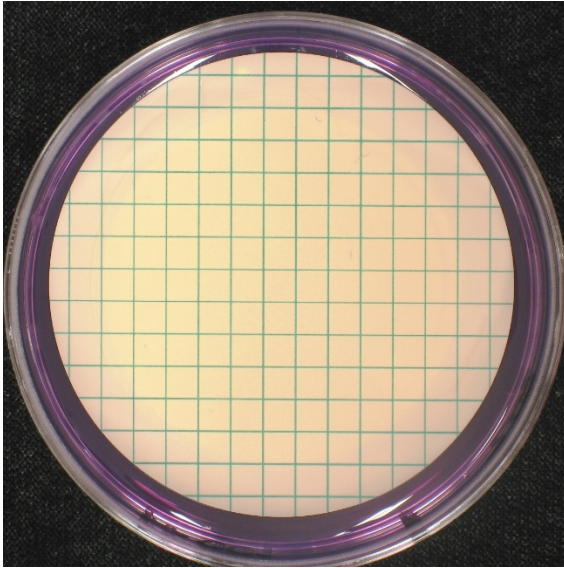
100 ml

**Chromocult Coliform Agar, 37 °C**



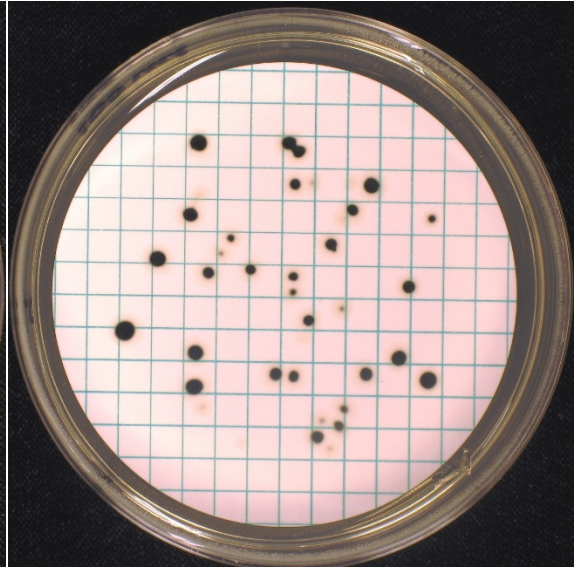
100 ml

**m-FC Agar, 44 °C**



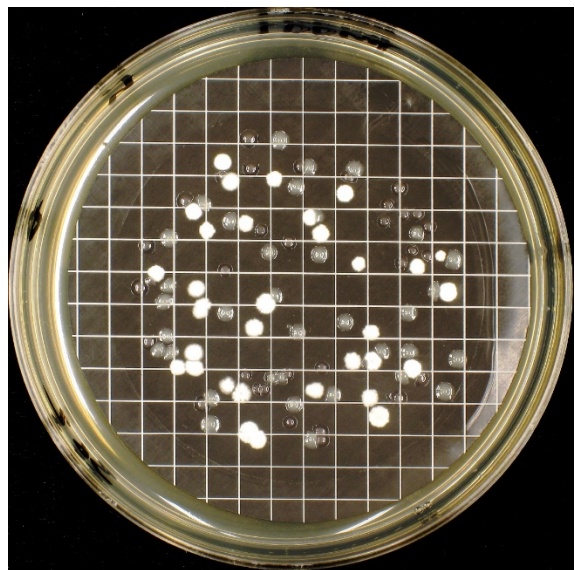
100 ml

**m-TSC Agar, 44 °C**



100 ml

**m-RBCC Agar, 25 °C**

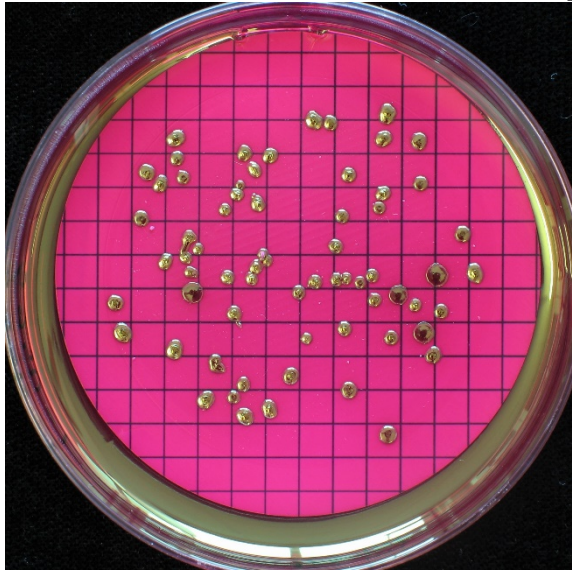


100 ml, 7 days

**Actinomycete Isolation Agar, 25 °C**

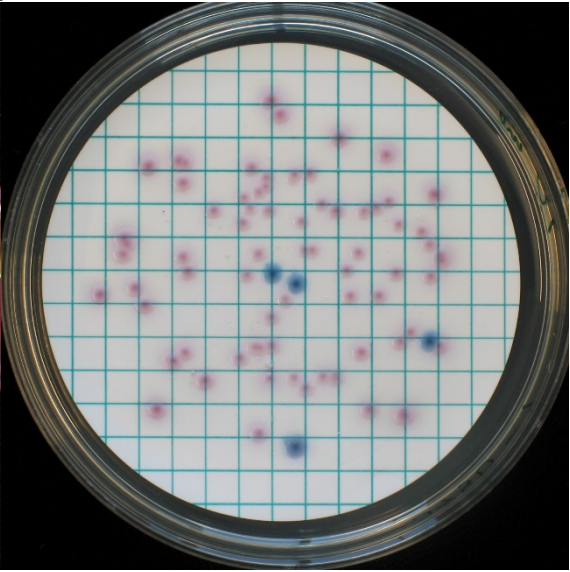
**Sample C**

**m-Endo Agar LES, 37 °C**



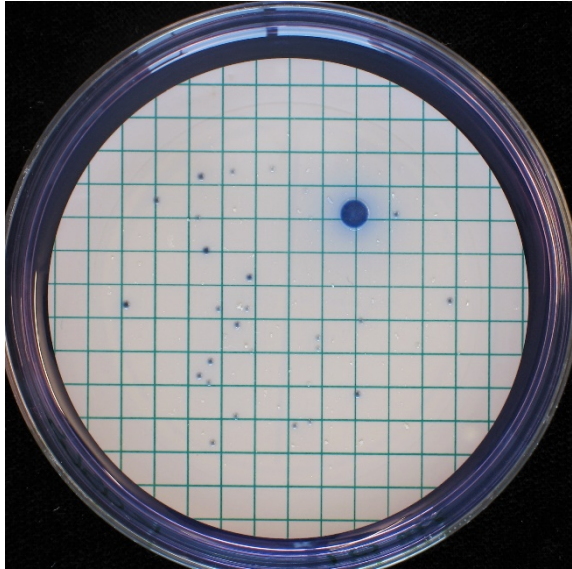
10 ml

**Chromocult Coliform Agar, 37 °C**



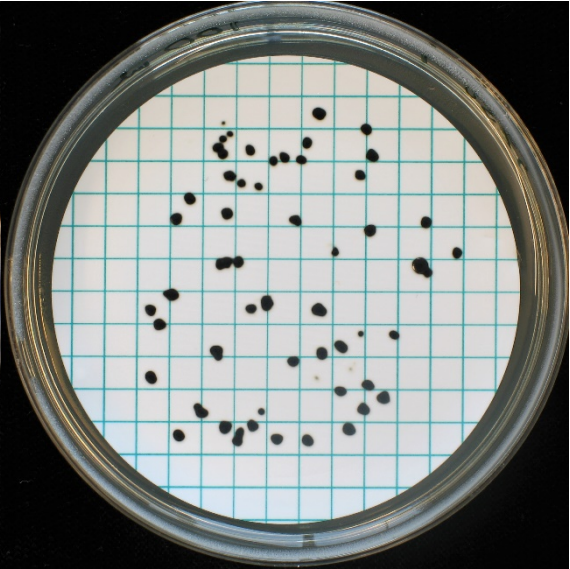
10 ml

**m-FC Agar, 44 °C**



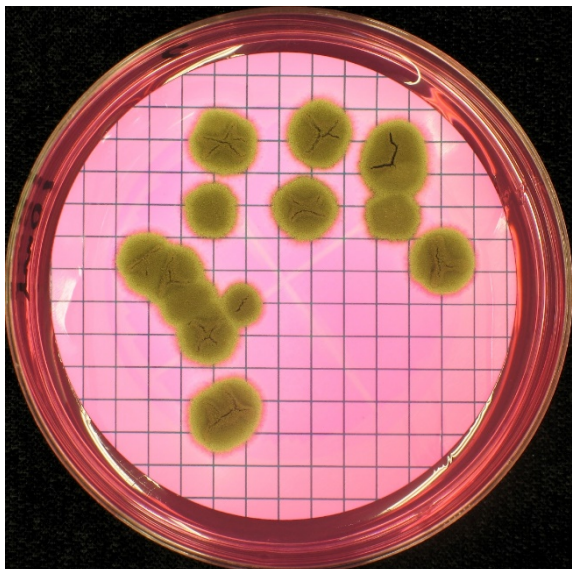
10 ml

**m-TSC Agar, 44 °C**



100 ml

**m-RBCC Agar, 25 °C**



100 ml, 7 days

**Actinomycete Isolation Agar, 25 °C**

## **PT reports published 2020**

Proficiency Testing – Food Microbiology, January 2020, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, March 2020, by  
Tommy Ślapokas

Proficiency Testing – Food Microbiology, April 2020, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, September 2020, by  
Linnea Blom and Tommy Ślapokas

Proficiency Testing – Food Microbiology, October 2020, by Jonas Ilbäck

## **PT reports published 2021**

Proficiency Testing – Food Microbiology, January 2021, 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>