

Drinking Water Microbiology

September 2020

Linnea Blom & Tommy Šlapokas



Edition

Version 1 (2020-12-03)

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 September 2020 is registered as no. 2020/02447 at the Swedish Food Agency, Uppsala

Proficiency testing
Drinking water Microbiology
September 2020

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±2 °C**

Culturable microorganisms (total count) 2 days incubation at **35/36/37 °C**

Abbreviations and explanations

Microbiological media

CCA	Chromocult Coliform Agar [®] (Merck; EN ISO 9308-1:2014)
Colilert	Colilert [®] Quanti-Tray [®] (IDEXX Inc.; EN ISO 9308-2:2014)
Enterolert	Enterolert [®] Quanti-Tray [®] (IDEXX Inc.)
LES	m-Endo Agar LES (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 [®] Quanti-Tray [®] (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

Abbreviations and explanations	2
Contents	3
General information on results evaluation	4
Results of the PT round	4
- General outcome	4
- Coliform bacteria (MF)	6
- Suspected thermotolerant coliform bacteria (MF)	8
- <i>Escherichia coli</i> (MF)	10
- Coliform bacteria and <i>E. coli</i> (rapid method, MPN)	12
- Intestinal enterococci (MF/MPN)	14
- <i>Pseudomonas aeruginosa</i> (MF/MPN)	16
- Culturable microorganisms 22 °C, 3 days	18
- Culturable microorganisms 36 °C, 2 days	20
Outcome of the results and laboratory assessment	22
- General information about reported results	22
- Base for assessment of the performance	22
- Mixed up results and other practical errors	22
- Z-scores, box plots and deviating results for each laboratory	22
Test material, quality control and processing of data	26
- Description of the test material	26
- Quality control of the test material	27
- Processing of numerical results	28
References	29
Annex A – All reported results	30
Annex B – Z-scores of the results	34
Annex C – Photo example of colony appearance on some media	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 28 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 89 laboratories, 34 in Sweden, 45 in other Nordic countries (Faeroe Islands, Greenland and Åland included), two more from EU, six from the rest of Europe and two from outside Europe. Results were reported from 86 laboratories.

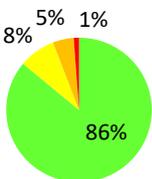
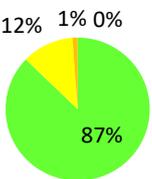
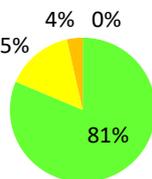
The percentages of false results and outliers are compiled in **table 1**.

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	493			489			496		
No. of deviating results *	18 (4 %)			12 (2 %)			19 (4 %)		
Microorganisms	<i>Escherichia coli</i> <i>Serratia marcescens</i> <i>Enterococcus hirae</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus saprophyticus</i>			<i>Escherichia coli</i> <i>Enterobacter aerogenes</i> <i>Enterococcus durans</i> <i>Burkholderia cepacia</i>			<i>Klebsiella pneumoniae</i> <i>Enterobacter cloacae</i> <i>Lactobacillus plantarum</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas fluorescens</i>		
	Analysis	Target org.	F %	X %	Target org.	F%	X%	Target org.	F%
Coliform bacteria (MF)	<i>E. coli</i> { <i>S. marcescens</i> }	2	2	<i>E. coli</i> <i>E. aerogenes</i>	0	3	<i>K. pneumoniae</i> <i>E. cloacae</i>	2	5
Susp. thermotolerant coliform bact. (MF)	<i>E. coli</i>	–	–	<i>E. coli</i> { <i>E. aerogenes</i> }	–	–	<i>K. pneumoniae</i> { <i>E. cloacae</i> }	–	–
<i>E. coli</i> (MF)	<i>E. coli</i>	5	0	<i>E. coli</i>	0	2	–	3	–
Coliform bacteria (rapid method)	<i>E. coli</i> <i>S. marcescens</i>	4	0	<i>E. coli</i> <i>E. aerogenes</i>	0	0	<i>K. pneumoniae</i> <i>E. cloacae</i>	2	0
<i>E. coli</i> (rapid meth.)	<i>E. coli</i>	5	2	<i>E. coli</i>	2	2	–	2	0
Intestinal enterococci (MF)	<i>E. hirae</i> { <i>S. saprophyticus</i> }	0	5	<i>E. durans</i>	0	5	{ <i>L. plantarum</i> }	2	0
<i>Pseudomonas aeruginosa</i> (MF)	<i>P. aeruginosa</i>	2	0	{ <i>B. cepacia</i> }	2	0	<i>P. aeruginosa</i>	2	0
Culturable micro-organisms (total count), 3 days	22 °C { <i>S. marcescens</i> (<i>E. hirae</i>) (<i>P. aeruginosa</i>) (<i>E. coli</i>) (<i>S. saprophyticus</i>)	0	3	<i>E. durans</i> <i>E. aerogenes</i> <i>E. coli</i> (<i>B. cepacia</i>)	0	3	(<i>E. cloacae</i>) (<i>K. pneumoniae</i>) (<i>P. aeruginosa</i>) (<i>L. plantarum</i>)	4	0
Culturable micro-organisms (total count), 2 days	36 °C { <i>S. marcescens</i> (<i>E. hirae</i>) (<i>P. aeruginosa</i>) (<i>E. coli</i>) (<i>S. saprophyticus</i>)	0	2	<i>E. durans</i> <i>E. aerogenes</i> <i>E. coli</i> (<i>B. cepacia</i>)	0	2	<i>P. fluorescens</i> (<i>E. cloacae</i>) (<i>K. pneumoniae</i>) (<i>P. aeruginosa</i>) (<i>L. plantarum</i>)	0	9

* In total 26 of 86 laboratories (30 %) 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)

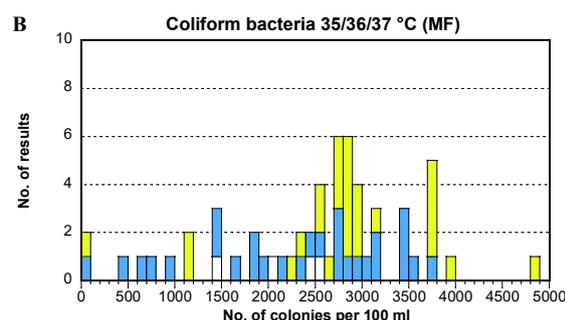
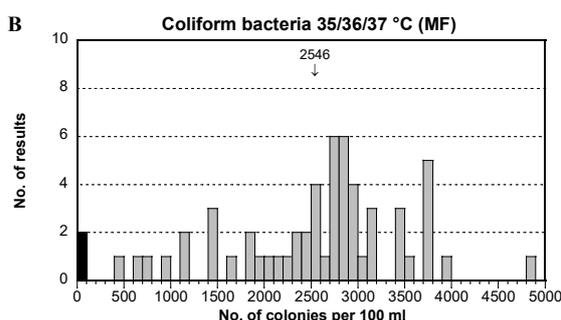
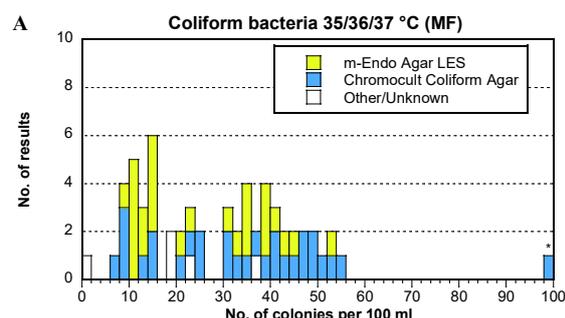
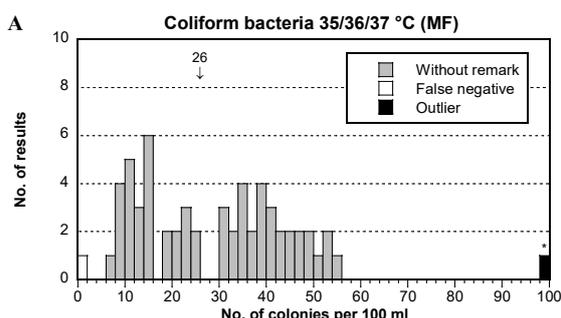
The primary cultivation media for the analysis of coliform bacteria were the enzyme-based chromogenic medium CCA and LES which is based on lactose fermentation. The group Other/Unknown in the table includes six different media, from both water and food methods, as well as from methods in the medical field.

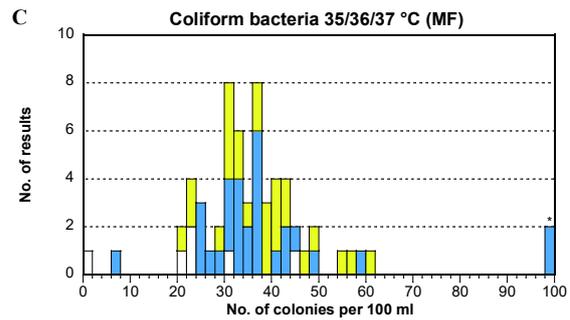
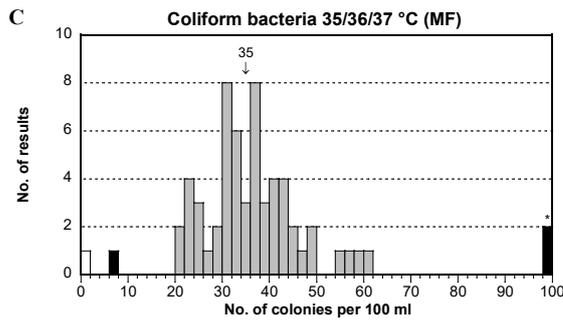
The average results for LES and CCA are more or less equivalent in samples A and C. However the results for CCA were somewhat lower compared to LES in sample B. The heterogenic group Other/Unknown contained false negative results in samples A and C.

In total six 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	<	>
Total	61	58	26	28	1	0	1	58	2546	21	0	2	0	57	35	13	1	1	2
m-Endo Agar LES	26	26	23	29	0	0	0	25	2819	15	0	1	0	26	37	14	0	0	0
Chromocult C. Agar	29	28	29	28	0	0	1	28	2312	26	0	1	0	26	35	10	0	1	2
Other/Unknown	6	4*	24	-	1	0	0	5	2559	24	0	0	0	5	27	17	1	0	0

*Mean value is given for comparison despite few results





Sample A

- The two coliform bacteria *Escherichia coli* and *Serratia marcescens* were included in the sample. The strain of *E. coli* appeared with for coliform bacteria characteristic colonies on the MF media at 37 °C, a metallic sheen on LES and blue on CCA. The other strain, *S. marcescens*, appears with small red colonies on LES that would normally not be considered as coming from a coliform bacterium. On CCA the colonies are fairly small and apricot coloured, indicating that they might come from a coliform bacterium.
- The results were distributed into two peaks, corresponding to the laboratories that excluded and included *S. marcescens*, respectively. This implies relatively high CV and thereby medium dispersion. Since the colonies *S. marcescens* do not appear with a metallic sheen on LES, it would be reasonable that laboratories using LES obtained lower results than those using CCA. The average result was also somewhat higher for CCA than LES. However, both media are present in both peaks.
- The average result for coliform bacteria was somewhat higher for rapid methods (Colilert®; page 12); 35 versus 26 cfu per 100 ml for the MF-methods, indicating that the strain of *S. marcescens* was detected to the full extent by the rapid methods. The average result for the rapid methods falls within the higher of the two peaks for the MF-method, where both coliform bacteria are assumed to be included.
- One high outlier and one false negative result were reported.

Sample B

- One strain each of *E. coli* and *Enterobacter aerogenes* were present as coliform bacteria in the sample. Both strains usually form characteristic colonies with MF methods at 37 °C. However, colonies of *E. aerogenes* do not always have a clear metallic sheen on LES, implying that they may not be interpreted as suspected coliform bacteria. Despite this, laboratories that used LES seem to have included *E. aerogenes* to full extent in this PT as the average result was somewhat higher for LES compared to CCA (see table). Since the bacterial background flora was low, the results rather suggest that one of the strains gave a lower yield on CCA, or had ambiguous coloured colonies that they were excluded.
- As in sample A, there was a tendency for somewhat higher average result with rapid methods (Colilert®; page 12); 3142 versus 2546 cfu per 100 ml.
- The distribution of the results was wide with medium dispersion (CV; see page 28). Two low outliers were present.

Sample C

- No *E. coli* but the coliform bacteria *Klebsiella pneumoniae* and *Enterobacter cloacae* were present in the sample. Both strains form characteristic colonies with MF methods at 37 °C.
- The distribution of the results was good with a small dispersion. Four deviating results were present. Two of the high outliers were obtained by CCA, which may indicate that colonies other than coliform bacteria were erroneously included.

Suspected thermotolerant coliform bacteria (MF)

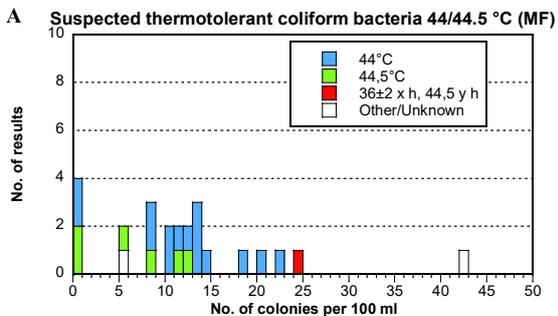
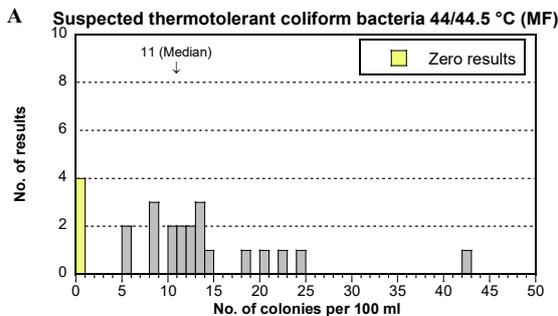
No evaluation in relation to performance is done for what is called suspected (not confirmed) colonies of the parameter. Therefore, no outliers are assessed. The *medians* are then more robust than the means and are given in the table and in histograms. **Thus, the parameter is not included in the performance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

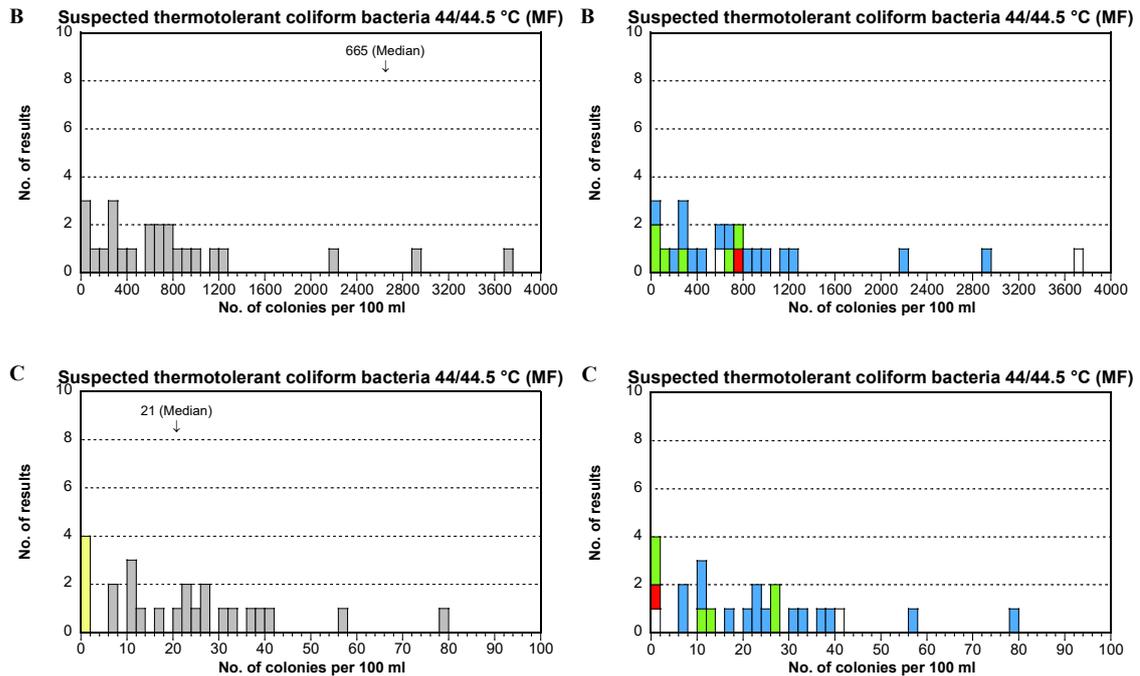
The primary growth media used at 44 or 44.5 °C to identify suspected thermotolerant coliform bacteria is m-FC. The two laboratories in the group Other/Unknown have stated methods where the primary media are incubated at 35/36/37 °C, and where 44 °C is used only for confirmation. This is not the intention 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. Most likely, the primary incubation at 35/36/37 °C without confirmation at high temperature is the cause for the high average for the group Other/Unknown, since there is no inhibitory effect due to high temperature.

Incubation temp.	N	A					B					C						
		n	Med	CV	F	<	>	n	Med	CV	F	<	>	n	Med	CV	F	<
Total	24	20	12	-	4	-	24	665	-	0	-	-	20	23	-	4	-	-
44 °C	15	13	13	-	2	-	15	710	-	0	-	-	15	22	-	0	-	-
44.5 °C	6	4*	10	-	2	-	6	194	-	0	-	-	4*	20	-	2	-	-
36 ± 2°C x h, 44.5°C y h	1	1	-	-	0	-	1	-	-	0	-	-	0	-	-	1	-	-
Other/Unknown	2	2*	24	-	0	-	2*	2165	-	0	-	-	1*	41	-	1	-	-

*Median is given for comparison despite few results

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





Sample A

- Two coliform bacteria were included in the sample; *E. coli* and *S. marcescens*. Of these, only the *E. coli* strain appears with characteristic blue colonies at 44/44.5 °C on m-FC.
- The median was 12 cfu/100 ml and there were four zero results. The strain of *E. coli* is gas negative. Gas production at 44/44.5 °C is in some standards a criterion for a strain to be included among the thermotolerant coliform bacteria. If this criterion has been used also when reporting suspected thermotolerant coliform bacteria – which is not in the definition of the parameter – it is plausible that the colonies from *E. coli* have not been reported.
- In Sweden, where incubation at 44 °C is used, gas production is not a criterion for a strain to be included among thermotolerant coliform bacteria. That gas production appears to have been used to some extent at 44.5 °C may explain the lower average results at this temperature. Also, the recovery of *E. coli* can be somewhat lower at 44.5 °C due to inhibition from high temperature.

Sample B

- Two coliform bacteria were included in the sample, of which only 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. aerogenes* may sometimes appear with small blue to grey colonies on m-FC, in particular if the temperature does not reach 44 °C.
- The median results are noticeably lower at 44.5 °C, likely because of inhibition due to the high temperature. The median for the group Other/Unknown was high because at least one of the laboratories included *E. aerogenes*, which was able to grow when the primary incubation temperature was 35/36/37 °C.

Sample C

- *K. pneumoniae* appears as brown-blue to blue colonies on m-FC agar at 44 °C. *E. cloacae* may also sometimes appear on m-FC with small blue colonies, which should then be added to the result for suspected thermotolerant bacteria.
- The four zero results indicate that those laboratories did not interpret the colonies as blue coloured and therefore did not include them as suspected thermotolerant coliform bacteria. Alternatively, the absence of gas production was the criterion for reporting 0 cfu/100 ml, or there were no colonies at all appearing at 44.5 °C.

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 β -glucuronidase activity from oxidase-negative presumptive strains are usually performed. A violet to blue colony on CCA indicates positive β -glucuronidase activity and is considered as a confirmed *E. coli*. Corresponding reactions occur on other chromogenic media based on β -glucuronidase activity.

The primary growth media CCA, LES and others are used at 35/36/37 °C and m-FC at 44/44.5 °C. In addition to primary 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 (NS, SS and SFS) the majority of the results are from incubation at 35/36/37 °C on LES but some are also from incubation at 44/44.5 °C on m-FC.

When comparing the results, there is a difference between the incubation temperatures for sample B. The average for m-FC was lower at 44/44.5 °C and the dispersion (CV) was very large. For the standards, there is an indication of a lower average for ISO 9308-1:2014 based on CCA compared to other groups in sample B.

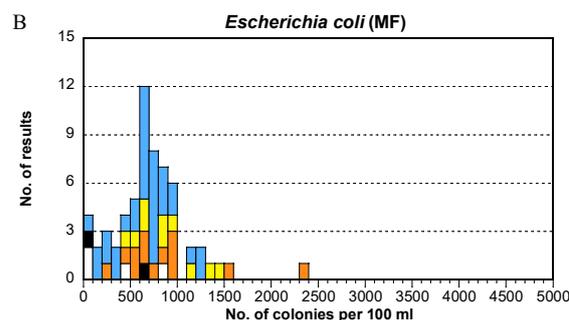
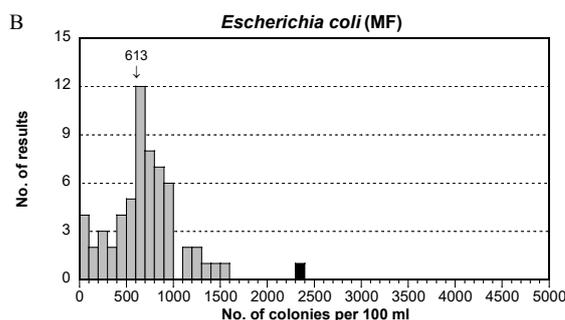
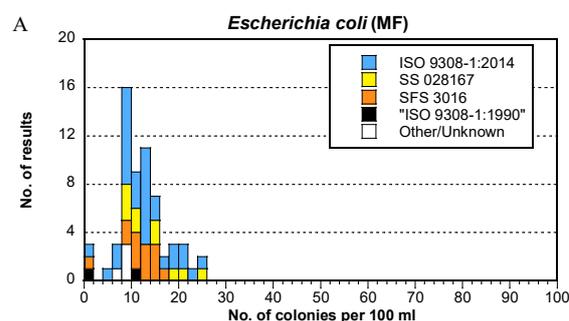
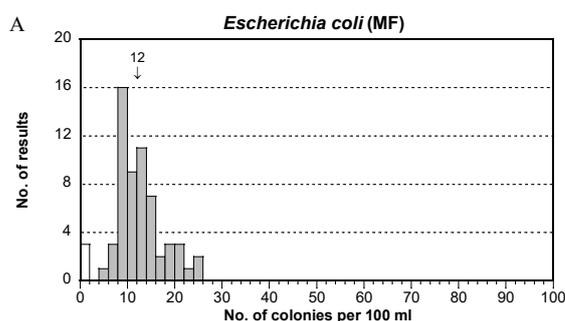
All results

Origin & Standard	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	62	58	12	18	3	0	0	60	613	31	0	0	1	60	0	-	2	-	-
<u>Colony origin</u>																			
36 ± 2 °C	44	42	13	18	1	0	0	42	720	20	0	0	1	43	0	-	1	-	-
44/44.5 °C	6	5	10	24	1	0	0	6	252	73	0	0	0	5	0	-	1	-	-
36 ± 2 & 44/44.5 °C	10	9	11	11	1	0	0	10	496	45	0	0	0	10	0	-	0	-	-
Other/Unknown	2	2	-	-	0	0	0	2	-	-	0	0	0	2	0	-	0	-	-
<u>Standard</u>																			
ISO 9308-1:2014	33	31	12	19	1	0	0	32	592	29	0	0	0	32	0	-	1	-	-
SS 028167	10	10	14	20	0	0	0	10	852	20	0	0	0	10	0	-	0	-	-
SFS 3016 (4088)	13	12	12	10	1	0	0	12	709	23	0	0	1	12	0	-	1	-	-
”ISO 9308-1:1990”	2	1	-	-	1	0	0	2	-	-	0	0	0	2	0	-	0	-	-
Other/Unknown	4	4	-	-	0	0	0	4	-	-	0	0	0	4	0	-	0	-	-

Results for *E. coli* 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	<	>
Total	49	46	12	19	2	0	0	47	670	26	0	0	1	47	0	-	2	-	-
m-Endo Agar LES	17	16	13	18	1	0	0	16	786	22	0	0	1	16	0	-	1	-	-
Chromocult C Agar	28	27	12	19	1	0	0	28	631	27	0	0	0	27	0	-	1	-	-
CCA, "wrong standard"	2	2	-	-	0	0	0	2	-	-	0	0	0	2	0	-	0	-	-
Other/Unknown	2	1	-	-	0	0	0	1	-	-	0	0	0	2	0	-	0	-	-

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



Sample A

- A strain of *E. coli* was included together with another coliform bacterium, *S. marcescens*. The colony appearance for *E. coli* is characteristic on LES and m-FC.
- The distribution of the results was good and the dispersion (CV) small. Three false negative results were present.
- The strain of *E. coli* is indole-positive and shows distinct β -glucuronidase activity, but does not produce gas in lactose broth at 44 °C. If gas production is a decisive criterion for a laboratory to detect *E. coli*, they should have reported a zero result, which may explain the three false negative results.

Sample B

- One typical *E. coli* strain was included together with another coliform bacterium, *E. aerogenes*. The *E. coli* strain is positive for β -glucuronidase activity, indole production and gas production. It forms typical colonies on the various primary

growth media. *E. aerogenes* is indole-negative and has no β -glucuronidase activity, meaning it cannot be mistaken for *E. coli* after confirmation.

- The distribution was inexplicably wide with a large dispersion (CV). As a consequence, the tail of low results were not regarded as low outliers. One high outlier was however reported.

Sample C

- No *E. coli* was included, however the coliform bacteria *K. pneumoniae* and *E. cloacae* were present in the sample. *K. pneumoniae* and sometimes even *E. cloacae* can grow at 44/44.5 °C. However, both strains are indole-negative, and have no activity of β -glucuronidase. They should therefore not be mistaken for *E. coli* after confirmation.
- Two false positive result were 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. Of the 56 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; β -galactosidase activity shown) will be interpreted as coliform bacteria and yellow wells also exhibiting fluorescence (MUG-positive; β -glucuronidase activity shown) will be interpreted as *E. coli*.

When trays with different number of wells as well as different incubation temperatures and incubation times were compared, the differences were small and inconsistent.

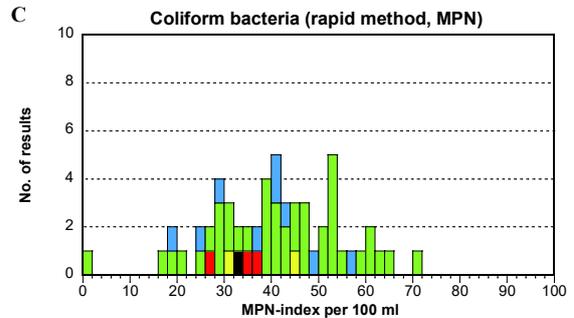
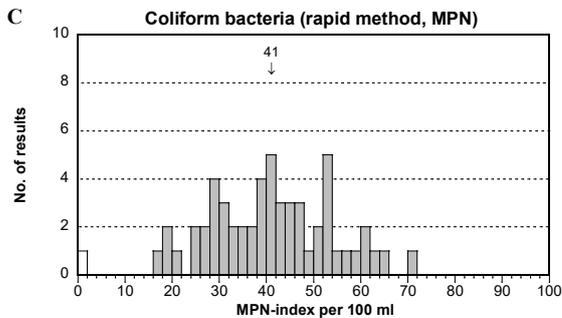
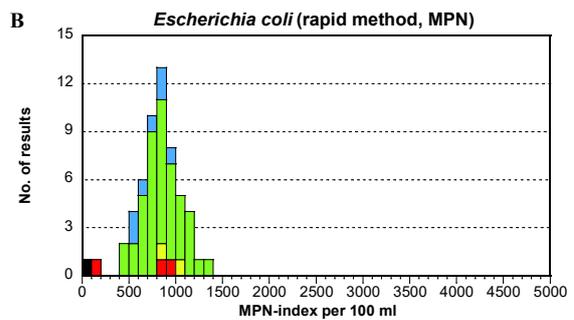
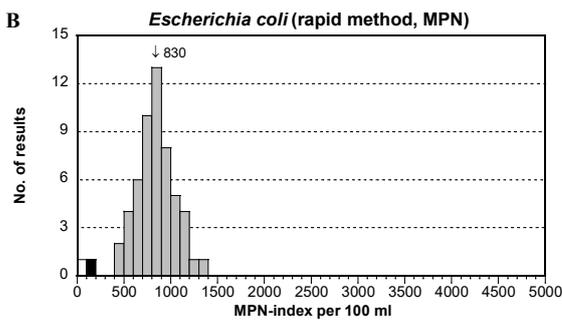
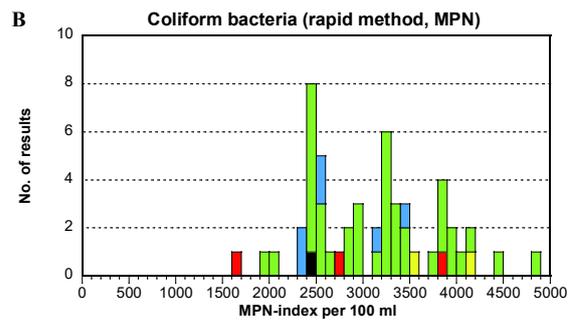
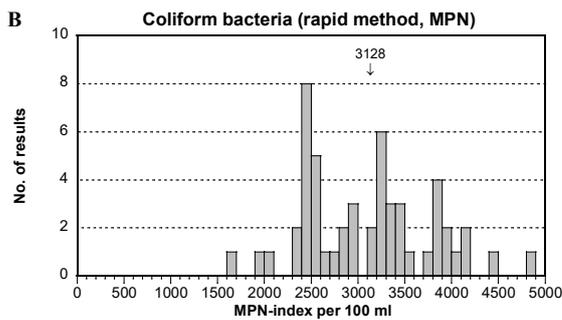
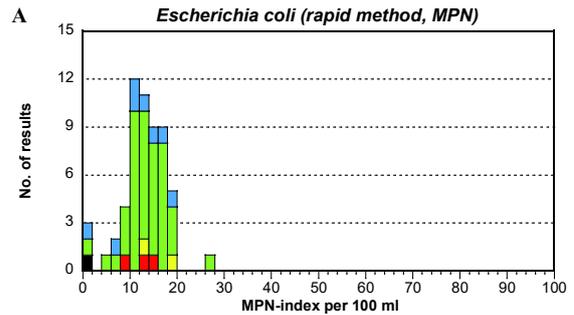
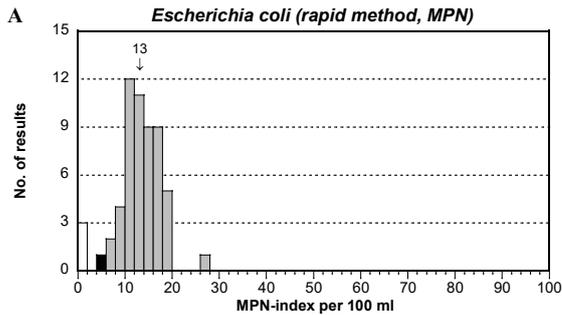
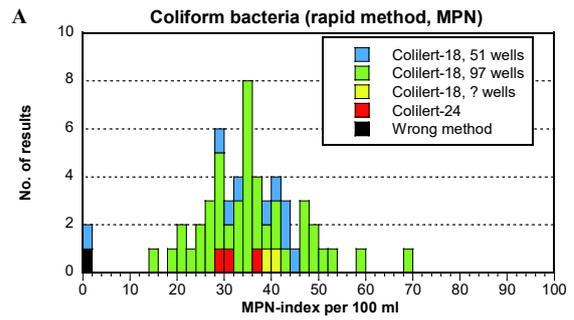
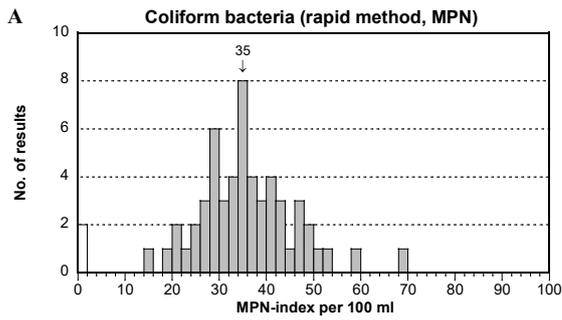
Coliform bacteria, Rapid method with MPN

Principle	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	56	55	35	14	1	0	0	53	3142	13	0	0	0	56	39	23	1	0	0
Colilert-18, 51 wells	9	8	37	9	1	0	0	6	2703	8	0	0	0	9	36	17	0	0	0
Colilert-18, 97 wells	42	42	35	16	0	0	0	42	3213	13	0	0	0	42	41	24	1	0	0
Colilert-18, 51 & 97	2	2	-	-	0	0	0	2	-	-	0	0	0	2	-	-	0	0	0
Colilert-24, ? wells	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0
Wrong method[#]	1	0	-	-	1	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	<	>
Total, Rapid meth	56	53	13	13	2	1	0	54	830	12	0	1	0	55	0	-	1	-	-
Colilert-18, 51 wells	8	7	12	17	1	0	0	7	725	11	0	0	0	8	0	-	0	-	-
Colilert-18, 97 wells	43	41	13	13	1	1	0	43	838	12	0	0	0	42	0	-	1	-	-
Colilert-18, 51 & 97	2	2	-	-	0	0	0	2	-	-	0	0	0	2	0	-	0	-	-
Colilert-24, ? wells	3	3	-	-	0	0	0	2	-	-	0	1	0	3	0	-	0	-	-
Wrong method*	1	0	-	-	1	0	0	0	-	-	1	0	0	1	0	-	0	-	-

[#] In this case no rapid kit method but a multiple tube method based on lactose fermentation.



Sample A

- The strains of *E. coli* and *S. marcescens* grow in the medium and possess the enzyme β -galactosidase. Therefore, they are detected as coliform bacteria by methods based on this enzyme (ONPG-positive) e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate.
- The strain of *E. coli* has the enzyme β -glucuronidase and is detected as *E. coli*.
- The distributions of the results were good and the dispersions small (CV; see p. 28). There were three false negative results and one low outlier for *E. coli* and two false negative result for coliform bacteria.
- The mean values 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 *E. aerogenes* grow in the medium and possess the enzyme β -galactosidase. Therefore, they are detected as coliform bacteria by methods based on this enzyme (ONPG-positive) e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate.
- *E. coli* has the enzyme β -glucuronidase and is detected as *E. coli*.
- The distribution of the results for coliform bacteria is not as dispersed as for the MF-method (lesser CV) and the average is higher.
- One low outlier and one false negative result were reported for *E. coli*.

Sample C

- No *E. coli* was present, but the coliform bacteria *K. pneumoniae* and *E. cloacae* were included in the sample. They both have the enzyme β -galactosidase and are thus detected as coliform bacteria. They however lack the enzyme β -glucuronidase and should therefore not be detected as *E. coli*.
- The average distribution of the results were somewhat wide with medium dispersion. One false negative result for coliform bacteria and one false positive result for *E. coli* were reported.
- The mean values for the accepted results for coliform bacteria were similar for the rapid method and the MF method (see p. 6).

Intestinal enterococci (MF/MPN)

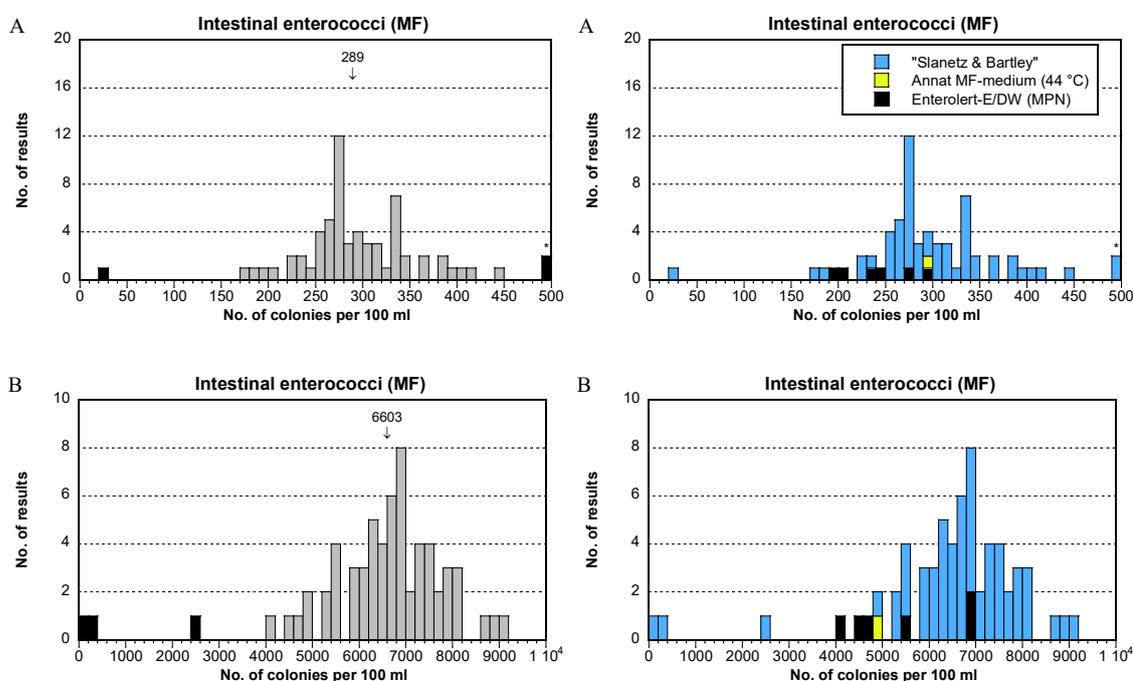
The MF method used for intestinal enterococci was almost exclusively EN ISO 7899-2:2000. The primary growth medium was m-Enterococcus Agar (Slanetz & Bartley), here designated m-Ent. One laboratory used Rapid Enterococcus Agar at 44 °C without confirmation. In the other seven cases the rapid method with Enterolert[®] (Idexx Inc.) was used. Five of these used Enterolert[®]-E (Idexx Inc.) and the other two used Enterolert[®]-DW (Idexx Inc.). The incubation temperature was 41 °C for the rapid method. The incubation temperature for m-Ent was 35, 36 or 37 °C, except for one laboratory that incubated at 41 °C.

In short, the most prominent method difference is the MF-method versus the rapid method. Somewhat lower mean values were seen for the rapid method. The dispersions were very small for all methods.

Method/Medium	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	C	F	<	>	n	Mv	CV	F	<	>
Total	65	61	289	9	0	1	2	61	6603	8	0	3	0	64	0	-	1	-	-
EN ISO 7899	55	52	295	9	0	1	2	52	6745	7	0	3	0	55	0	-	0	-	-
Slanetz & Bartley	57	54	295	9	0	1	2	54	6783	7	0	3	0	57	0	-	0	-	-
Other/Unknown	1	1	-	-	0	0	0	1	-	-	0	0	0	1	0	-	0	-	-
Rapid method [#] , MPN	7	6	240	8	0	0	0	6	5361	11	0	0	0	6	0	-	1	-	-

*Mean is given for comparison despite few results

Two variants of Enterolert[®], E and DW, respectively– no confirmation was performed



Sample A

- A strain of *Enterococcus hirae* was included in the sample. It forms typical red to brown colonies on m-ENT agar that are usually confirmed as enterococci without problem.
- One low and three high outliers were present.
- The results by Enterolert[®] are somewhat lower than those by the MF-method.

Sample B

- A strain of *Enterococcus durans* was included in the sample. It appeared as typical red to brown colonies on m-ENT.
- The distribution of the results was good with very small dispersion.

- Three low outliers were reported.
- The results by Enterolert[®] were lower than those by m-Ent.

Sample C

- No intestinal enterococcus strain was included in the sample.
- One false positive result was reported.

Pseudomonas aeruginosa (MF/MPN)

EN ISO 16266:2008 with or without modification was used by 39 of the 50 laboratories that reported results. Pseudalert[®] (Idexx Inc.) was used by nine laboratories.

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

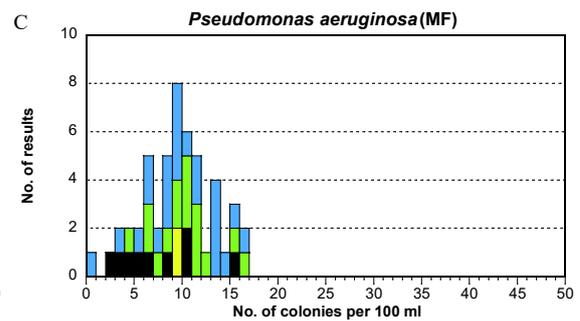
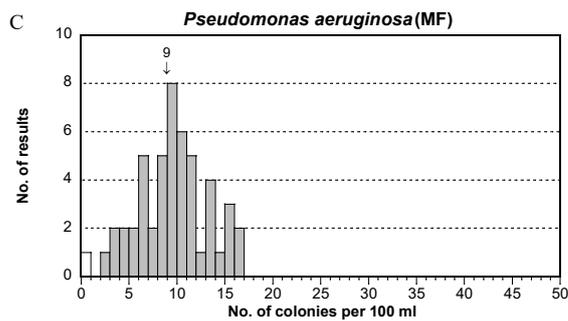
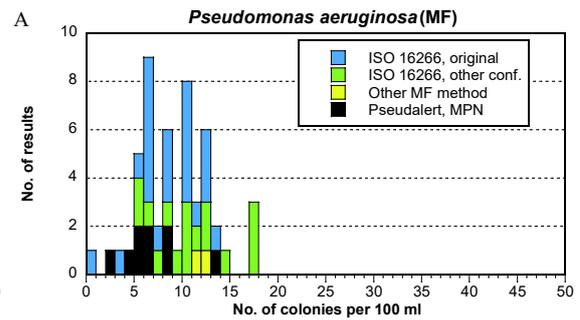
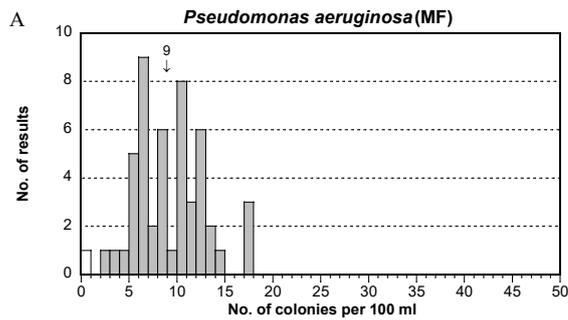
The colonies in sample A were typical, meaning no confirmation was necessary. The colonies in sample C were not completely typical, as they had both a blue-green and a red-brown pigment on PACN. The brownish colour is most easily distinguished when viewed from the bottom of the plate. It can also be seen for colonies that are transferred to an unselective medium. For both sample A and C, the colonies were clearly fluorescing in UV light.

The average results for Pseudalert[®] seemed to be somewhat lower with larger dispersion (CV) compared to the MF-methods. However, the concentrations of the target bacteria were low in both samples making this comparison rather uncertain.

Standard/Method	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	50	49	9	21	1	0	0	49	0	-	1	-	-	49	9	21	1	0	0
Membrane filtration	41	40	9	19	1	0	0	40	0	-	1	-	-	40	9	17	1	0	0
ISO 16266 ^a	23	22	8	17	1	0	0	22	0	-	1	-	-	22	10	19	1	0	0
ISO 16266, mod. ^b	16	16	10	20	0	0	0	16	0	-	0	-	-	16	9	17	0	0	0
Other	2	2	-	-	0	0	0	2	0	-	0	-	-	2	-	-	0	0	0
Pseudalert [®] , MPN	9	9	6	25	0	0	0	9	0	-	0	-	-	9	6	31	0	0	0

a Modification not stated for confirmation

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



Sample A

- *Pseudomonas aeruginosa* was present in the sample. On PACN agar it forms blue-green colonies that fluoresce under UV-light. No confirmation was therefore required according to the standard EN ISO 16266:2008.
- The distribution of the results was good with medium dispersion (CV; see page 28), primarily due to the low concentration in the sample (≤ 10 cfu / 100 ml).
- One false negative results was reported.

Sample B

- No *P. aeruginosa* was present in the sample but *Burkholderia cepacia* formed yellowish colonies on PACN. Some laboratories reported these as suspected *P. aeruginosa* and one laboratory reported them as confirmed *P. aeruginosa*. The other laboratories reporting "presumptives" correctly obtained a negative outcome in their confirmation.

Sample C

- A strain of *Pseudomonas aeruginosa* was present in the sample. On PACN agar it forms atypical brownish-green colonies that fluoresce under UV-light. Because of the greenish pigment and fluorescence on PACN, no confirmation of the colonies was needed according to the standard EN ISO 16266:2008.
- When confirmation is performed it verifies that the colonies are *P. aeruginosa*.
- The distribution of the results was good with medium dispersion. The dispersion for Pseudalert[®] is considered as large (CV, see page 28). The reason being, as in sample A, the low concentration of *P. aeruginosa* (≤ 10 cfu/100 ml).
- There was one false negative result.

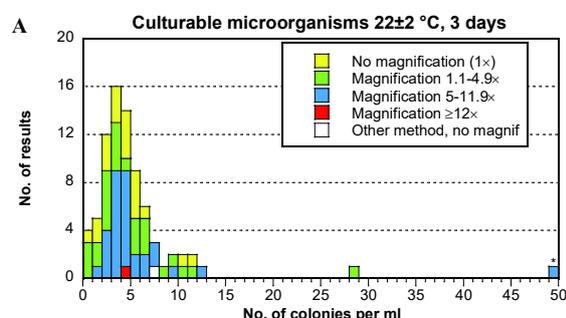
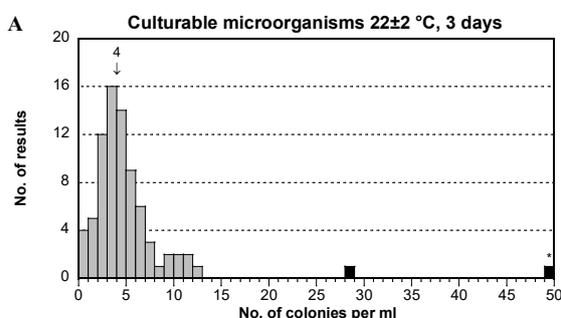
Culturable microorganisms 22 °C, 3 days

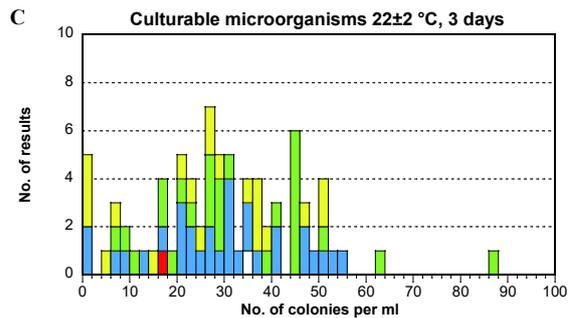
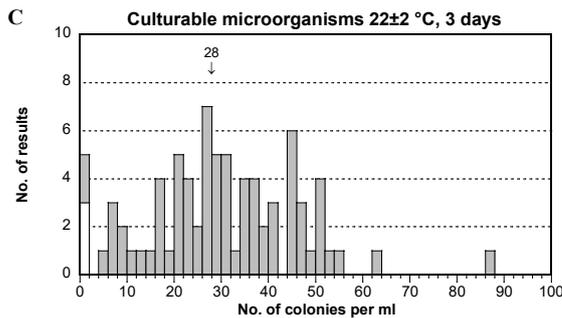
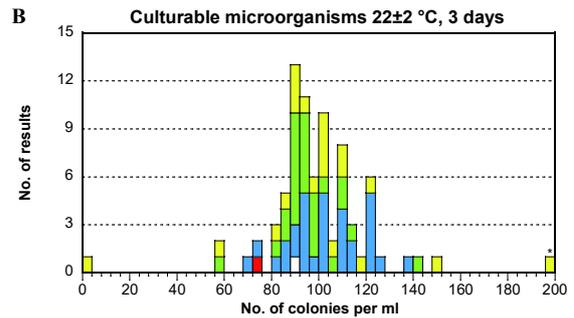
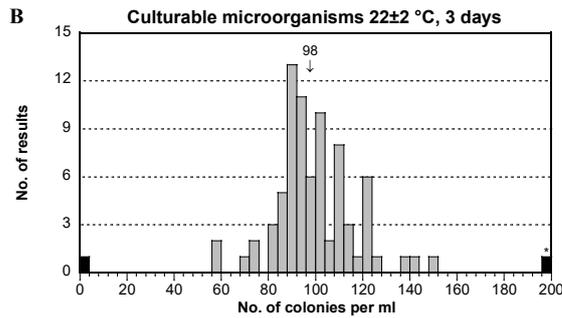
Seventy-seven of the 79 laboratories performing the analysis reported following EN ISO 6222:1999, which prescribes the use of Yeast extract Agar (YeA). Ten laboratories used Plate Count Agar instead, simultaneously stating the use of EN ISO 6222:1999. One laboratory used Petrifilm™ and another laboratory used YeA in conjunction with Standard methods [5]. These laboratories comprises the group “Other method”. The majority of the laboratories have claimed counting both bacterial and fungal colonies. Ten laboratories stated that they did not count fungi, and four stated that they counted 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 difference based on methods or relation to magnification between the samples. Plate Count Agar tended to give a larger dispersion (CV) than YeA. There were no small colonies present that could be difficult to discern. This may explain why there were no major differences when different magnifications were used for counting.

Group of results	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	79	77	4	37	0	0	2	77	98	8	0	1	1	79	28	29	3	0	0
EN ISO 6222	77	75	4	37	0	0	2	75	98	8	0	1	1	74	28	29	3	0	0
<i>Medium</i>																			
Yeast extract Agar	67	66	4	36	0	0	1	65	99	7	0	1	1	65	28	29	2	0	0
"Plate Count Agar"	10	9	3	50	0	0	1	10	97	14	0	0	0	9	25	35	1	0	0
<i>Magnification</i>																			
None	20	19	4	35	0	0	0	17	99	9	0	1	1	17	25	34	2	0	0
1,1–4,9×	26	25	3	52	0	0	1	26	95	8	0	0	0	26	31	28	0	0	0
5–11,9×	31	30	4	26	0	0	1	31	102	8	0	0	0	30	28	28	1	0	0
≥ 12×	1	1	–	–	0	0	0	1	–	–	0	0	0	1	–	–	0	0	0
Other method	2	2	–	–	0	0	0	2	–	–	0	0	0	2	–	–	0	0	0





Sample A

- The few colonies originate from all strains in the sample.
- The dispersion of the results was large. This is normal when the concentration is low (<10 cfu/1ml).
- There were two high outliers.

Sample B

- All four strains constitute the culturable microorganisms in proportion to their concentration.
- The distribution was good and the dispersion (CV) was very small.
- One low and one unreasonably high outlier were reported.

Sample C

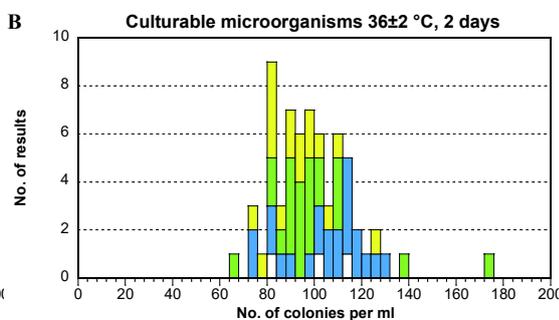
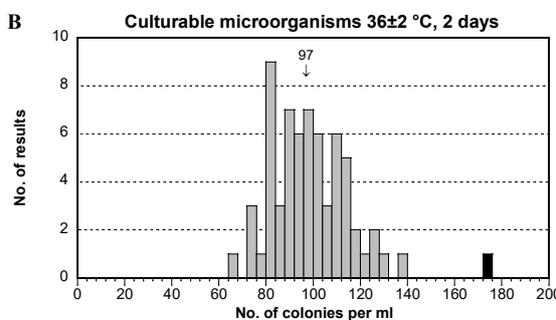
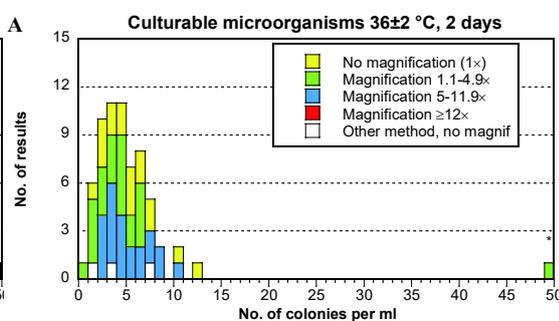
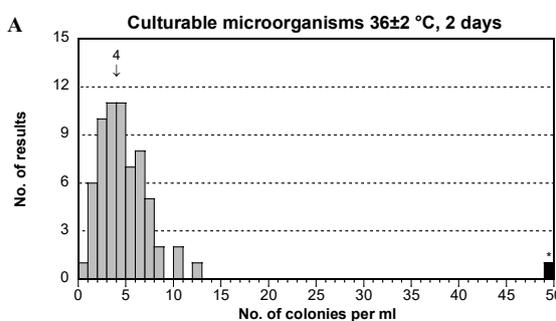
- The colonies consist almost exclusively of *Pseudomonas fluorescens*. The other strains will also grow, but appear in very low numbers.
- The distribution was not good and showed medium dispersion. The strain of *P. fluorescens* is known to give quite scattered distributions, even though the colonies are not particularly small.
- Due to the many low results it was impossible to discern any outliers. However, three zero results were present, which here are classified as false negatives.

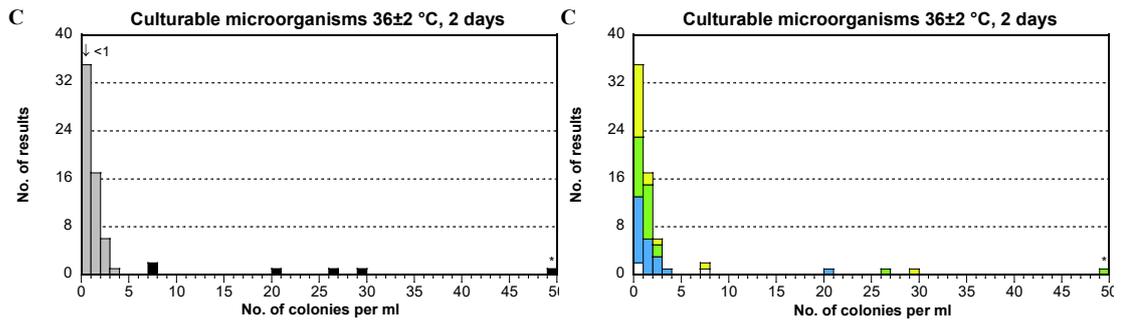
Culturable microorganisms 36 °C, 2 days

Sixty-two of the 65 laboratories followed EN ISO 6222:1999. Six of these reported incubating on Plate Count Agar (PCA). The values for PCA are for comparison shown in parallel with YeA for EN ISO 6222:1999 in the table below. One of the three laboratories in the group Other/Unknown stated the use of Standard Methods [5].

As for the analysis at 22 °C, comparisons of method variants are relevant to discuss only when EN ISO 6222:1999 was used. Here as well, results are shown for both culture media and for magnification at reading. No general differences were however seen for either of these groups.

Group of results	N	A					B					C								
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	
Total, all results	65	64	4	32	0	0	1	64	97	8	0	0	0	1	59	0	–	0	0	6
<i>EN ISO 6222</i>	62	61	4	32	0	0	1	61	97	8	0	0	0	1	57	0	–	0	0	5
<i>Medium</i>																				
Yeast extract Agar	56	55	4	31	0	0	1	55	98	8	0	0	0	1	51	0	–	0	0	5
Plate Count Agar	6	6	6	26	0	0	0	6	85	7	0	0	0	0	6	0	–	0	0	0
<i>Magnification</i>																				
None	17	17	5	31	0	0	0	17	92	7	0	0	0	0	15	0	–	0	0	2
1,1–4,9×	23	22	3	37	0	0	1	22	95	7	0	0	1	21	0	–	0	0	0	2
5–11,9×	22	22	4	25	0	0	0	22	103	8	0	0	0	21	0	–	0	0	0	1
Other/Unknown	3	3	–	–	0	0	–	3	–	–	0	0	0	0	2	–	–	0	0	1





Sample A

- All strains included in the sample appeared with a few colonies as culturable microorganisms at 35/36/37 °C. Due to the low numbers of colonies, the dispersion was large although the distribution was still good.
- Due to the very low average number of colonies, even a result of zero cfu per ml is reasonable and acceptable.
- One unreasonably high extreme value was reported.

Sample B

- Colonies from all strains in the sample appeared as culturable microorganisms at 35/36/37 °C, in proportion to the individual strain concentrations.
- The distribution of the results was good with a very small dispersion (CV; see page 28). One high outlier was reported.

Sample C

- The strain *P. fluorescens* that was included in the sample does not grow at 35/36/37 °C. Due to low concentrations, the other strains will appear only in very low numbers, <1 cfu per ml in total. Zero results are therefore both expected and acceptable.
- The distribution of the results was good except for six high outliers.

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

Twenty-six laboratories have more than one deviating result. When whole samples appear have been mixed up, the corresponding sample numbers are crossed out in annex A. No laboratory appears to have mixed up whole samples. However, two laboratories (1235 and 8955) may have mixed up the samples for some of the parameters. One laboratory reported their results as \log_{10} values, which contradicts the instruction for the PT round.

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

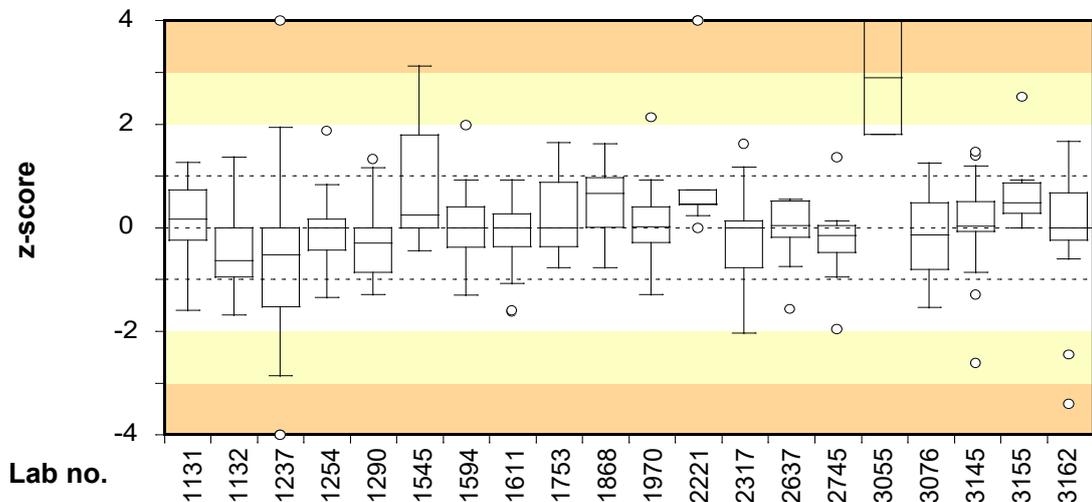
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are reported in annex B but are not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control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

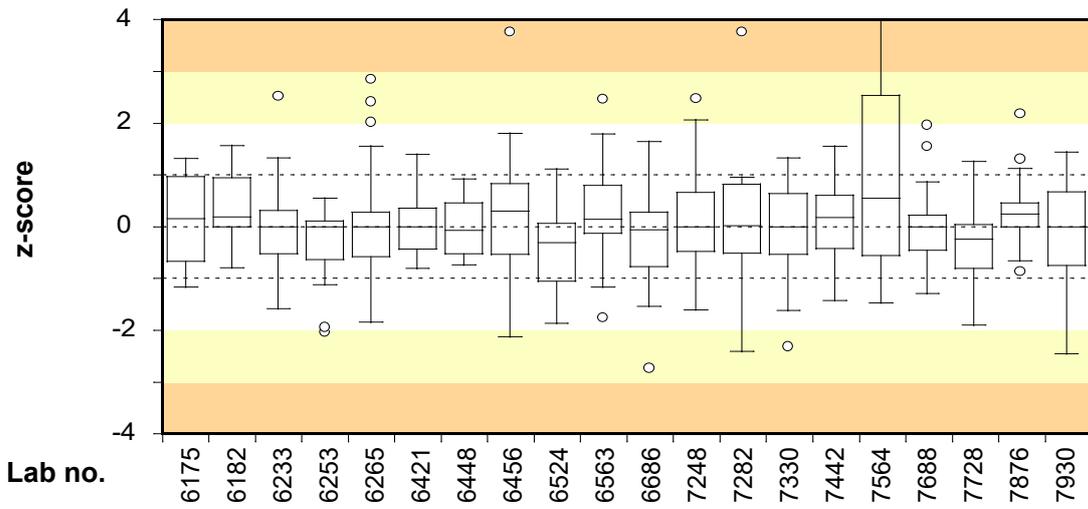
Box plots and numbers of deviating results for each participating laboratory

- 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.

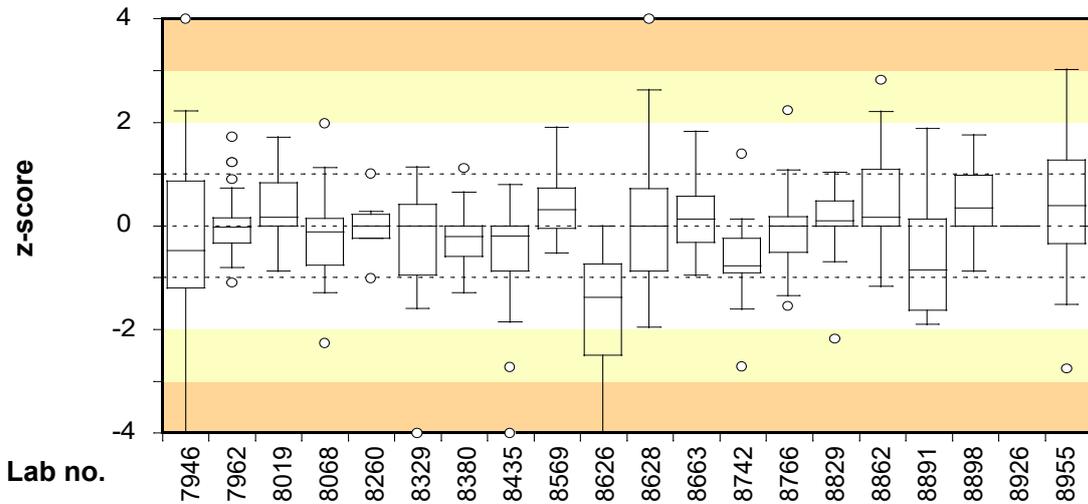
* $< [\text{smallest value of the box} - 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$ or $> [\text{largest value of the box} + 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$



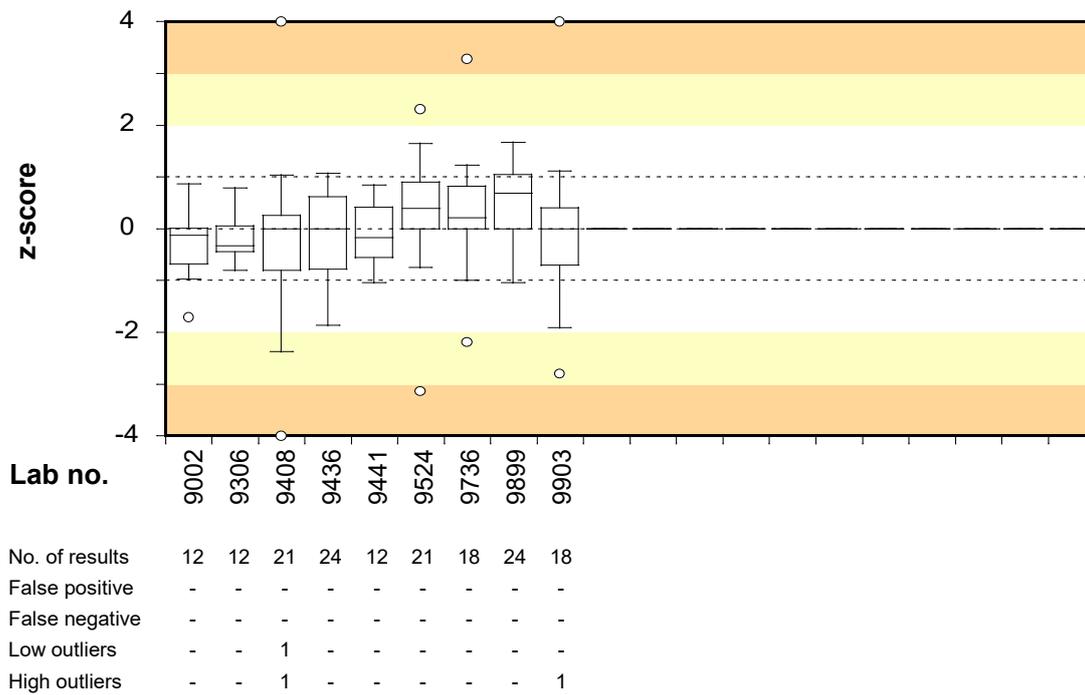
Lab no.	1131	1132	1237	1254	1290	1545	1594	1611	1753	1868	1970	2221	2317	2637	2745	3055	3076	3145	3155	3162
No. of results	9	9	21	24	18	24	24	24	18	15	18	9	18	12	9	2	9	15	8	18
False positive	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
False negative	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Low outliers	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
High outliers	-	-	2	-	-	-	-	-	-	-	-	2	-	-	-	1	-	-	-	-



Lab no.	6175	6182	6233	6253	6265	6421	6448	6456	6524	6563	6686	7248	7282	7330	7442	7564	7688	7728	7876	7930	
No. of results	10	18	24	11	19	18	6	14	12	24	10	24	12	12	15	12	24	18	21	20	
False positive	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	5	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	2	-	-	-	-	-



Lab no.	7946	7962	8019	8068	8260	8329	8380	8435	8569	8626	8628	8663	8742	8766	8829	8862	8891	8898	8926	8955	
No. of results	21	24	23	24	9	18	24	18	18	8	18	24	12	24	9	24	5	24	-	20	
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
False negative	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	2
Low outliers	1	-	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-
High outliers	3	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1



Test material, quality controls and processing of data

Description of the test material

This PT round comprised three test items with different microorganism compositions. The test material was manufactured and freeze-dried in 0.5 ml portions 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 participants were instructed to perform the analyses according to the methods routinely used at their laboratories.

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 ¹	Microorganism	Strain collection no.		cfu/100 ml ²
		SLV ³	Reference ⁴	
A	<i>Escherichia coli</i>	532	CCUG 48891	15
	<i>Serratia marcescens</i>	040	ATCC 13 880	35
	<i>Enterococcus hirae</i>	536	CCUG 46536	300
	<i>Pseudomonas aeruginosa</i>	453	CCUG 551	20
	<i>Staphylococcus saprophyticus</i>	013	CCUG 45100	<1*
B	<i>Escherichia coli</i>	082	CCUG 45097	1000
	<i>Enterobacter aerogenes</i>	099	ATCC 13 048	3500
	<i>Enterococcus durans</i>	078	CCUG 44816	7500
	<i>Burkholderia cepacia</i>	042	–	150
C	<i>Klebsiella pneumoniae</i>	537	–	10
	<i>Enterobacter cloacae</i>	187	CCUG 43599	30
	<i>Lactobacillus plantarum</i>	475	CCUG 30503	<1
	<i>Pseudomonas aeruginosa</i>	569	–	15
	<i>Pseudomonas fluorescens</i>	535	CCUG 45106	65*

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 Internal strain collection number at the Swedish Food Agency (SLV).

4 Origin or typing collection no., CCUG: Culture Collection University of Gothenburg, ATCC: American Type Culture Collection; A dash (–) indicates a strain from the Swedish Food Agency's internal culture collection that has not yet been characterised at another culture collection.

Quality control of the test material

In order to allow comparison of results from the freeze-dried samples, it is essential that the original sample mixture is homogeneous and that a uniform volume is distributed in all vials. The sample volume was monitored during production by weighing 2-3 % of the vials before and after addition of the sample. The largest detected differences between vials were 7, 8 and 5 mg in samples A, B and C, respectively. The largest accepted difference is 15 mg (3 %).

Table 3 Concentration (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 ¹								
	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>	54	1,0	1,3	45 ^b	0,7	1,3	44	1,2	1,4
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar. 44 °C according to SS 028167</i>	12 ^d	–	1,5	6 ^b	1,7	2,5	16	1,0	1,7
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	17	1,4	1,8	9 ^b	1,0	1,8	–	–	–
Intestinal enterococci (MF) <i>m-Enterococcus Agar acc. to SS-EN ISO 7899-2:2000</i>	29 ^a	1,1	1,5	75 ^b	1,6	1,3	–	–	–
<i>Pseudomonas aeruginosa</i> (MF) <i>Pseudomonas Agar base with cetrimide and nalidixic acid according to SS-EN ISO 16266:2008</i>	21	1,3	1,6	–	–	–	16	1,1	1,7
Culturable microorg. 2d 37 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	3 ^b	0,9	3,7	103 ^b	1,2	1,3	0,3 ^b	1,7	
Culturable microorg. 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	3 ^b	0,5	2,1	101 ^b	1,1	1,2	66 ^b	1,9	1,4

1 10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, analysed 24, 21 and 16 weeks ahead of the testing round for samples A, B and C, respectively.

a Determined for the volume 10 ml

b Determined for the volume 1 ml

c Determined for the volume 50 ml

d m-FC was analysed without duplicates, therefore the homogeneity cannot be assessed

– No target organism and thus no analysis

Table 3 shows 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. The values are from duplicate analyses of 10 vials the first time a sample mixture is used or from duplicate analyses of 5 vials when a sample 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 sample mixtures were homogeneous with regard to the parameters that were 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, \log_{10} transformation of the results is normally not routine. Instead, for the low concentrations normally encountered here, square root transformations of the results usually 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 the 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, for example when many zero results are reported and in some borderline cases, subjective adjustments are made based on the knowledge of the sample mixture's content in order to set the correct 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, the measurement uncertainty will also 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. It is here provided as the relative uncertainty (u_{rel}), which is expressed as per cent after division by the mean value mv and multiplication by 100.

More information about result processing and recommendations on follow-up analyses are provided 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, 5th 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*).

Annex A Results of the participants. Susp. = suspected on membrane filter before confirmation. Results given as <1, <2, <10 and <100 are treated as zero. The fields with other results given as < 'value' and results given as > 'value' are yellow, and those results are not included in calculations or evaluations. This is also valid for results in shaded columns. A hyphen indicate that no result has been reported. Figures written in bold in yellow fields indicate outliers, false positive and false negative results. Underlined zero values indicate results characterized as 'False negative?'. Crossed out sample numbers in a row indicate that the samples probably are mixed up. False positive and false negative values

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	21	3260	46	12	980	0
1132	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	29	2420	20	10	1120	0
1237	2	1	3	-	-	-	21	8	1300	-	-	-	<1	8	450	30	>2400	24	11	690	<1
1254	1	2	3	10	2900	32	10	2900	32	-	-	-	10	970	0	37	2910	28	12	980	0
1290	1	2	3	-	-	-	32	1817	25	-	-	-	9	270	<1	-	-	-	-	-	-
1545	2	3	1	20	3700	55	20	3700	55	20	590	11	20	590	0	58	3255	39	26	839	0
1594	1	2	3	-	-	-	35	2800	30	10	425	7	11	950	0	37	2800	24	14	665	0
1611	2	1	3	35	2500	22	35	2500	22	-	-	-	14	769	0	33	1986	28	11	770	0
1753	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	47	3870	53	12	1150	0
1868	2	1	3	-	-	-	48	3400	42	-	-	-	20	657	0	35	3873	58	11	685	0
1970	2	3	1	11	2900	57	11	2900	57	11	2900	57	11	670	0	-	-	-	-	-	-
2221	1	2	3	-	-	-	35	3400	600	-	-	-	13	800	0	-	-	-	-	-	-
2317	1	2	3	-	-	-	46	1900	36	-	-	-	20	660	0	-	-	-	-	-	-
2637	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	28	3100	49	12	550	0
2745	1	3	2	50	2390	27	50	2390	27	5	630	0	5	630	0	-	-	-	-	-	-
3055	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	50	2481	44	14	980	0
3155	1	2	3	-	-	-	-	-	-	8	127	11	-	-	-	-	-	-	-	-	-
3162	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	15	3255	51	4	789	0
3305	3	1	2	42	2800	30	39	2800	30	-	-	-	12	500	<1	31	2000	53	14	620	<1
3587	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3730	3	2	1	17	1400	14	-	-	-	13	850	20	-	-	-	-	-	-	-	-	-
3883	3	2	1	31	3181	48	31	3181	48	-	-	-	9	618	<1	35	3772	47	16	616	<1
4015	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	35	3300	47	14	990	0
4288	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4339	2	3	1	34	2200	33	34	2200	33	22	270	38	9	660	0	33	3448	36	11	980	0
4343	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	20	2595	39	11	1120	0
4356	3	2	1	12	2800	30	12	2800	30	8	390	6	12	933	0	35	3466	17	14	866	0
4459	1	3	2	21	1482	22	19	1482	22	-	-	-	9	645	<1	28	1664	35	9	189	<1
4635	1	3	2	-	-	-	>1	>1	<1	-	-	-	>1	>1	<1	-	-	-	-	-	-
4723	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	28	3873	63	11	1046	0
4889	3	2	1	-	-	-	47	3000	25	-	-	-	16	660	0	34	3900	28	16	980	0
5018	2	1	3	33	2700	23	33	2700	21	-	-	-	17	270	0	47	2420	38	12	816	0
5094	3	1	2	-	-	-	9	2490	31	-	-	-	9	710	0	-	-	-	-	-	-
5220	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	33	2420	18	8	727	0
5352	2	1	3	-	-	-	48	3450	31	-	-	-	13	950	0	-	-	-	-	-	-
5447	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	28	2500	0	17	530	0
5858	2	3	1	-	-	-	14	1435	36	-	-	-	12	265	<1	-	-	-	-	-	-
5950	1	3	2	52	1155	60	52	1155	60	14	1036	17	19	891	<1	35	2513	42	7	1036	<1
6175	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	38	>200	56	18	>200	<1
6182	2	1	3	28	2633	35	41	3733	35	-	-	-	13	1100	<1	52	3932	30	16	910	<1
6233	1	2	3	8	2700	32	8	2700	32	-	-	-	8	1200	0	24	5475	41	13	840	0
6253	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	40	2300	40	15	800	0
6265	2	1	3	30	2500	30	23	2500	30	12	1154	22	12	114	0	0	2400	33	0	0	0
6421	1	2	3	-	-	-	31	2100	32	0	260	0	15	773	0	-	-	-	-	-	-
6448	2	3	1	-	-	-	19	2400	44	-	-	-	9	800	0	-	-	-	-	-	-
6456	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	43	2380	43	16	530	<1
6524	3	1	2	-	-	-	7	1800	35	-	-	-	7	110	<1	-	-	-	-	-	-
6563	1	2	3	42	3700	41	42	3700	41	42	3700	41	25	1480	<1	40	4100	44	16	717	<1
6686	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	<1	2540	40.6	<1	885	<1
7248	3	2	1	10	1100	41	10	1100	41	10	900	37	10	1100	<1	41	2420	34	12	1120	<1
7282	1	2	3	-	-	-	-	-	-	-	-	-	8	40	0	-	-	-	-	-	-
7330	2	3	1	-	-	-	-	-	-	-	-	-	6	50	0	-	-	-	-	-	-
7442	1	2	3	-	-	-	54	3167	44	-	-	-	19	700	0	24	3277	45	11	573	0
7564	2	1	3	-	-	-	36	2000	23	-	-	-	9	410	0	-	-	-	-	-	-
7688	1	2	3	22	2800	37	22	2800	37	-	-	-	22	540	0	26	3300	53	11	870	0
7728	3	2	1	-	-	-	9	900	36	-	-	-	9	700	0	-	-	-	-	-	-
7876	1	2	3	15	2700	38	15	2700	38	-	-	-	15	600	<1	39	3332	33	18	762	<1
7930	1	2	3	42	2900	33	42	2900	33	-	-	-	8	850	<1	43	>2000	18	6	700	<1
7946	1	2	3	24	1410	58	24	1410	58	18	1225	79	10	446	0	27	2420	60	11	435	0
7962	1	3	2	41	2600	42	41	2600	42	13	310	24	13	600	0	48	2610	40	12	1203	0
8019	3	1	2	41	3100	42	41	3100	42	11	770	27	15	940	0	45	2540	29	18	885	0
8068	2	3	1	-	-	-	15	2300	37	-	-	-	15	500	0	26	4100	31	9	730	0
8260	1	3	2	24	2750	33	24	2750	33	-	-	-	8	700	0	-	-	-	-	-	-
8329	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	23	3475	55	10	802	0
8380	1	2	3	-	-	-	11	2850	31	-	-	-	11	855	0	35	3100	27	11	665	0
8435	3	2	1	-	-	-	39	2700	31	5	3	12	12	530	0	-	-	-	-	-	-
8569	1	3	2	38	2530	45	38	2530	41	-	-	-	15	430	0	42	4838	40	16	730	0
8626	2	3	1	12	54	27	12	54	22	0	54	22	0	54	0	-	-	-	-	-	-
8628	1	2	3	-	-	-	280	6200	25	0	23	0	10	790	0	-	-	-	-	-	-
Mean							26	2546	35				12	613	0	35	3128	41	13	830	0
CV (%)							28	21	13				18	31	-	14	13	18	13	12	-

are excluded, as well as other outliers, in the summarizing calculated results at the end of the table. The mean value (Mean) is the square of the mean value for the square root transformed results (mv). The coefficient of variation (CV) is the standard deviation (s) in percentage of the mean value for the square root transformed results. As means to calculate the z-values of your own, the appropriate values of mv and s are given at the end of the table. The x-values are obtained as the square roots of the reported result, respectively. $z = (x - mv) / s$. $u_{rel,mv}$ is the relative standard uncertainty of mv in per cent. For calculation see the scheme protocol (1); also briefly described in the text.

Susp. intestinal enterococci (MF)			Intestinal enterococci (MF)			Susp. Pseudomonas aeruginosa (MF)			Pseudomonas aeruginosa (MF)			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	
-	-	-	-	-	-	-	-	-	-	-	-	3	120	51	-	-	-	1131
-	-	-	238	6867	0	-	-	-	-	-	-	-	-	-	-	-	-	1132
-	-	-	570	330	<1	-	-	-	8	<1	4	5	87	5	10	75	2	1237
400	6200	0	400	6200	0	9	0	9	9	0	9	4	110	22	2	82	0	1254
-	-	-	273	7900	<1	-	-	-	8	<1	6	8	94	11	4	81	<1	1290
300	8900	0	300	8900	0	17	0	10	17	0	10	9	96	22	10	112	2	1545
330	5550	0	330	5550	0	17	0	10	17	0	10	4	98	37	2	92	1	1594
272	5500	0	272	5500	0	10	0	11	10	0	11	3	101	28	4	91	1	1611
260	6400	0	260	6400	0	-	-	-	6	0	10	2	92	32	3	119	2	1753
-	-	-	-	-	-	7	0	8	-	-	-	7	102	40	-	-	-	1868
250	6800	0	250	6800	0	10	0	9	10	0	9	1	94	45	6	81	1	1970
340	8200	0	-	-	-	5	0	13	-	-	-	-	-	-	5	104	29	2221
-	-	-	290	5400	0	-	-	-	6	0	3	6	83	37	3	99	0	2317
-	-	-	320	7200	0	-	-	-	-	-	-	5	100	36	-	-	-	2637
320	7200	0	-	-	-	-	-	-	-	-	-	4	91	21	-	-	-	2745
-	-	-	-	-	-	-	-	-	-	-	-	10	350	0	-	-	-	3055
-	-	-	-	-	-	-	-	-	12	0	14	5	85	26	1	88	0	3076
-	-	-	291	4106	0	-	-	-	8	0	15	1	103	51	-	-	-	3145
-	-	-	310	6800	<1	-	-	-	10	45	11	-	-	-	6	139	1	3155
290	6400	0	290	6400	0	12	0	16	12	0	16	3	89	28	6	91	1	3162
270	6600	<1	270	6600	<1	6	<1	11	6	<1	11	6	91	63	3	83	<1	3305
-	-	-	-	-	-	-	-	-	-	-	-	66	110	34	-	-	-	3587
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	113	1	3730
263	6727	<1	263	6727	<1	8	<1	15	8	<1	15	6	88	<1	-	-	-	3883
314	6800	0	286	6700	0	-	-	-	-	-	-	3	111	36	-	-	-	4015
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4288
310	6600	0	249	6870	0	8	0	10	6	0	6	5	110	16	5	110	1	4339
291	5800	0	273	5800	0	-	-	-	8	0	2	3	109	27	2	130	0	4343
220	6000	0	220	6000	0	-	-	-	5	0	6	3	88	17	4	109	0	4356
282	5200	<1	282	5200	<1	-	-	-	-	-	-	2	94	8	2	92	1	4459
>1	>1	<1	-	-	-	-	-	-	>1	-	-	0.54	1.96	1.15	-	-	-	4635
252	6000	0	252	6000	0	-	-	-	-	-	-	4	126	53	-	-	-	4723
-	-	-	310	7500	0	-	-	-	6	0	6	2	90	26	1	86	0	4889
410	6300	0	410	6300	0	11	80	8	11	0	8	4	138	30	4	72	0	5018
-	-	-	330	6210	0	-	-	-	-	-	-	3	94	6	2	72	0	5094
-	-	-	276	>2420	0	-	-	-	2	0	3	3	90	7	6	103	0	5220
-	-	-	330	6200	0	-	-	-	10	0	13	1	95	30	5	126	1	5352
340	7200	0	340	7200	0	-	-	-	-	-	-	2	110	50	5	90	1	5447
175	4825	<1	175	4825	<1	-	-	-	5	<1	8	11	91	41	4	97	1	5858
272	8600	<1	272	8600	<1	14	<1	15	14	<1	15	5	123	46	6	124	<1	5950
-	-	-	-	-	-	-	-	-	-	-	-	7	88	35	3	80	<1	6175
365	6733	<1	365	6733	<1	-	-	-	-	-	-	3	101	26	-	-	-	6182
340	8100	0	340	8100	0	-	-	-	5	0	10	2	98	29	1	102	1	6233
-	-	-	190	4700	1	-	-	-	-	-	-	4	100	28	-	-	-	6253
380	6000	0	380	6000	0	40	250	11	0	0	11	11	150	0	12	80	0	6265
-	-	-	269	7000	0	-	-	-	6	0	7	4	87	31	8	85	1	6421
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6448
-	-	-	>201	4530	<1	-	-	-	-	-	-	10	90	45	5	110	7	6456
270	5500	<1	-	-	-	-	-	-	-	-	-	4	93	24	7	100	<3	6524
-	-	-	202	5418	<1	7	0	12	7	0	12	3	102	29	4	105	<1	6563
6	5400	<1	-	-	-	-	-	-	-	-	-	<1	88	45	1	95	2	6686
440	9000	<1	440	9000	<1	10	<1	10	10	<1	10	3	110	20	4	110	<1	7248
334	7550	0	334	7550	0	11	0	9	11	0	9	-	-	-	1	112	7	7282
275	8100	0	275	8100	0	12	0	9	12	0	9	-	-	-	7	103	0	7330
-	-	-	-	-	-	7	0	22	-	-	-	2	95	40	-	-	-	7442
-	-	-	-	-	-	-	-	-	-	-	-	5	142	87	6	173	92	7564
380	7000	0	380	7000	0	-	-	-	5	0	9	1	97	44	4	87	0	7688
-	-	-	270	5300	0	-	-	-	8	0	9	2	120	15	7	92	0	7728
255	6900	<1	255	6900	<1	13	<1	13	13	<1	13	-	-	-	5	104	3	7876
230	6700	<1	230	6700	<1	8	<1	5	-	-	-	3	112	20	6	120	<1	7930
240	3858	0	225	2420	0	1	0	4	-	-	-	28	58	44	684	64	26	7946
360	5800	0	360	5800	0	6	0	8	6	0	8	3	93	27	4	81	0	7962
390	6400	0	390	6400	0	12	0	0	12	0	0	4	115	31	2	97	0	8019
-	-	-	180	6200	0	-	-	-	17	0	6	2	104	39	4	98	0	8068
-	-	-	-	-	-	-	-	-	-	-	-	3	103	47	-	-	-	8260
300	44	0	300	23	0	-	-	-	5	0	4	2	115	49	4	115	0	8329
265	6450	0	265	6450	0	11	0	7	11	0	7	1	91	25	7	89	0	8380
-	-	-	25	6900	0	6	0	5	6	0	5	0	96	6	2	96	0	8435
292	7300	0	264	7300	0	-	-	-	-	-	-	3	120	54	-	-	-	8569
-	-	-	-	-	-	-	-	-	-	-	-	0	82	22	-	-	-	8626
-	-	-	330	7500	0	-	-	-	3	0	13	4	90	13	2	78	0	8628
			289	6603	0				9	0	9	4	98	28	4	97	0	Mean
			9	8	-				21	-	21	37	8	29	32	8	126	CV (%)

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
8663	3 1 2	15	3700	42	14	3700	38	13	710	33	14	1500	0	32	2800	71	14	710	0
8742	3 1 2	-	-	-	14	450	28	-	-	-	10	330	<1	-	-	-	-	-	-
8766	2 1 3	10	2700	28	10	2700	28	0	200	10	10	920	0	37	3260	43	12	870	0
8829	1 3 2	-	-	-	36	730	36	-	-	-	12	380	0	-	-	-	-	-	-
8862	3 2 1	59	2818	37	44	2818	37	-	-	-	18	818	0	35	5794	61	18	1320	0
8891	1 2 3	-	-	-	<1	5000	20	-	-	-	-	-	-	-	-	-	-	-	-
8898	3 2 1	39	4818	45	39	4818	39	-	-	-	9	1364	0	40	4055	53	17	835	0
8926	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8955	2 1 3	-	-	-	45	2900	47	8	2200	31	0	2300	14	68	4400	65	0	770	31
9002	3 1 2	-	-	-	23	1600	37	-	-	-	9	400	0	-	-	-	-	-	-
9306	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	36	2755	36	12	902	0
9408	2 3 1	-	-	-	30	620	6	-	-	-	6	620	<1	39	3500	31	13	1046	<1
9436	1 3 2	8	3700	42	8	3700	42	12	700	27	8	645	0	19	2910	38	9	1025	0
9441	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	31	3840	27	15	885	<1
9524	2 3 1	59	2500	41	53	2500	41	24	750	<1	24	750	<1	28	2920	50	19	882	<1
9736	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	48	2472	102	16	456	0
9899	2 1 3	13	3500	48	13	3500	48	-	-	-	13	1200	0	47	3282	52	15	1027	0
9903	3 1 2	-	-	-	14	3900	35	-	-	-	9	830	<1	-	-	-	-	-	-

n	34	34	34	60	60	61	24	24	24	61	61	62	57	54	57	57	56	57
Min	8	54	14	0	8	0	0	3	0	0	8	0	0	1664	0	0	0	0
Max	59	4818	60	280	6200	1300	42	3700	79	25	2300	450	68	5794	102	26	1320	31
Median	26	2700	37	30.5	2700	35	11	665	21	11.5	660	0	35	3177.5	40.5	12	839.5	0
Mean				26	2546	35				12	613	0	35	3128	41	13	830	0
CV (%)				28	21	13				18	31	-	14	13	18	13	12	-
False positive				0	0	0				0	0	2	0	0	0	0	0	1
False negative				1	0	1				3	0	0	2	0	1	3	1	0
Outliers, low				0	2	1				0	0	0	0	0	0	1	1	0
Outliers, high				1	0	2				0	1	0	0	0	0	0	0	0
Low limit OK	8	54	14	7	450	20	0	3	0	5	8	10*	15	1664	17	6	435	0
High limit OK	59	4818	60	54	6200	60	42	3700	79	25	1500	0	68	5794	102	26	1320	0

mv				5.102	50.457	5.922				3.459	24.758	0.000	5.909	55.925	6.378	3.614	28.801	0.000
($\sqrt{\text{Mean}}$)																		
s				1.439	10.774	0.764				0.624	7.666	0.000	0.832	7.157	1.135	0.475	3.409	0.000
($CV \cdot mv/100$)																		
$u_{rel,mv}$ (%)				3.7	2.8	1.7				2.4	4.0		1.9	1.7	2.4	1.8	1.6	
($100 \cdot s / \sqrt{n_{mv}} / mv$)																		
x																		
($\sqrt{\text{Result}}$)																		
z																		
($(x-mv)/s$)																		

Susp. intestinal enterococci (MF)			Intestinal enterococci (MF)			Susp. <i>Pseudomonas aeruginosa</i> (MF)			<i>Pseudomonas aeruginosa</i> (MF)			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	
250	7800	0	250	7800	0	10	0	10	10	0	10	9	110	26	5	92	0	8663
-	-	-	-	-	-	-	-	-	-	-	-	4	87	8	8	89	<1	8742
270	5900	0	270	5900	0	7	0	13	7	0	13	12	75	20	3	114	0	8766
-	-	-	-	-	-	-	-	-	-	-	-	5	116	34	-	-	-	8829
273	6700	0	273	6700	0	-	-	-	13	0	8	4	101	31	3	80	1	8862
-	-	-	-	-	-	-	-	-	-	-	-	4	74	16	-	-	-	8891
279	6909	0	279	6909	0	12	0	11	12	0	11	4	120	23	2	100	2	8898
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8926
-	-	-	330	8100	0	-	-	-	4	0	5	5	59	26	3	90	1	8955
-	-	-	290	4900	0	-	-	-	-	-	-	3	98	44	-	-	-	9002
-	-	-	-	-	-	-	-	-	-	-	-	6	92	20	3	93	0	9306
-	-	-	1240	7600	<1	5	<1	5	-	-	-	2	84	18	1	100	<1	9408
318	7800	0	318	7800	0	6	0	8	6	0	8	7	103	17	6	82	0	9436
207	>2005	<1	-	-	-	-	-	-	-	-	-	2	93	39	3	97	<1	9441
280	7400	<1	280	7400	<1	-	-	-	-	-	-	6	105	51	<1	111	2	9524
336	7727	0	336	7727	0	10	0	9	10	0	9	6	83	34	2	98	1	9736
276	7300	0	276	7300	0	12	0	16	12	0	16	5	121	46	3	116	1	9899
-	-	-	300	6900	<1	-	-	-	10	<1	9	2	70	1	3	110	20	9903

47	46	47	64	64	65	33	32	33	50	50	50	79	79	79	65	65	65	n
6	44	0	25	23	0	1	0	0	0	0	0	0	1.96	0	0	64	0	Min
440	9000	0	1240	9000	1	40	250	22	17	45	16	66	350	87	684	173	92	Max
282	6700	0	280	6727	0	10	0	10	8	0	9	4	96	29	4	97	0	Median
			289	6603	0				9	0	9	4	98	28	4	97	0	Mean
			9	8	-				21	-	21	37	8	29	32	8	126	CV (%)
			0	0	1				0	1	0	0	0	0	0	0	0	False pos.
			0	0	0				1	0	1	0	0	3	0	0	0	False neg.
			1	3	0				0	0	0	0	1	0	0	0	0	Outliers <
			2	0	0				0	0	0	2	1	0	1	1	6	Outliers >
6	44	0	175	4106	0	1	0	0	2	0	2	0	58	1	0	64	0	Low limit
440	9000	0	440	9000	0	40	250	22	17	0	16	12	150	87	12	139	3	High limit

			17.013	81.258	0.000				2.918	0.000	2.976	1.902	9.924	5.300	1.955	9.841	0.461	mv
			1.595	6.581	0.000				0.608	0.000	0.612	0.699	0.815	1.536	0.622	0.772	0.579	s
			1.2	1.0					3.0		2.9	4.2	0.9	3.3	4.0	1.0	16.4	u _{rel,mv} (%)
																		x
																		z

Annex B z-scores calculated from the laboratory results. *Susp.* = Suspected on the membrane filters before confirmation. $z = (x - mv) / s$. z-scores are calculated also for outliers (excluding false negative results) in the same way as ordinary z-scores. From false

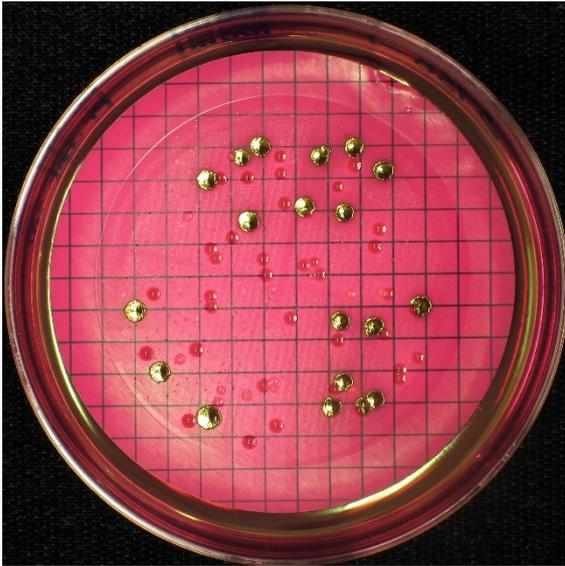
Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2	1	3																		
1132	2	1	3																		
1237	2	1	3																		
1254	1	2	3																		
1290	1	2	3																		
1545	2	3	1																		
1594	1	2	3																		
1611	2	1	3																		
1753	2	1	3																		
1868	2	1	3																		
1970	2	3	1																		
2221	1	2	3																		
2317	1	2	3																		
2637	1	2	3																		
2745	1	3	2																		
3055	3	2	1																		
3076	3	1	2																		
3145	2	3	1																		
3155	1	2	3																		
3162	2	1	3																		
3305	3	1	2																		
3587	3	1	2																		
3730	3	2	1																		
3883	3	2	1																		
4015	1	2	3																		
4288	1	2	3																		
4339	2	3	1																		
4343	2	1	3																		
4356	3	2	1																		
4459	1	3	2																		
4635	1	3	2																		
4723	2	1	3																		
4889	3	2	1																		
5018	2	1	3																		
5094	3	1	2																		
5220	1	3	2																		
5352	2	1	3																		
5447	1	2	3																		
5858	2	3	1																		
5950	1	3	2																		
6175	2	1	3																		
6182	2	1	3																		
6233	1	2	3																		
6253	2	3	1																		
6265	2	1	3																		
6421	1	2	3																		
6448	2	3	1																		
6456	1	3	2																		
6524	3	1	2																		
6563	1	2	3																		
6686	1	3	2																		
7248	3	2	1																		
7282	1	2	3																		
7330	2	3	1																		
7442	1	2	3																		
7564	2	1	3																		
7688	1	2	3																		
7728	3	2	1																		
7876	1	2	3																		
7930	1	2	3																		
7946	1	2	3																		
7962	1	3	2																		
8019	3	1	2																		
8068	2	3	1																		
8260	1	3	2																		
8329	2	1	3																		
8380	1	2	3																		
8435	3	2	1																		
8569	1	3	2																		
8626	2	3	1																		
8628	1	2	3																		
8663	3	1	2																		
8742	3	1	2																		
8766	2	1	3																		
8829	1	3	2																		
8862	3	2	1																		
8891	1	2	3																		
8898	3	2	1																		
8926	2	1	3																		
8955	2	1	3																		
9002	3	1	2																		
9306	2	3	1																		
9408	2	3	1																		
9436	1	3	2																		

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
9441	1 2 3																		
9524	2 3 1				1.513	-0.042	0.629				2.307	0.343	0.000	-0.411	0.844	-1.041	0.545	0.278	0.000
9736	2 1 3													-0.743	-0.264	0.611	1.568	0.263	0.000
9899	2 1 3				-1.040	0.808	1.316				0.235	1.289	0.000	1.225	-0.867	3.281	0.812	-2.185	0.000
9903	3 1 2				-0.945	1.113	-0.008				-0.734	0.529	0.000	1.138	0.191	0.735	0.545	0.952	0.000
n		0	0	0	59	60	60	0	0	0	58	61	60	55	54	56	54	55	56
Min					-1.707	-4.000	-4.000				-1.958	-2.861	0.000	-2.448	-2.114	-1.987	-3.397	-4.000	0.000
Max					4.000	2.625	4.000				2.469	3.026	0.000	2.809	2.821	3.261	3.126	2.209	0.000
Median					0.323	0.140	0.047				-0.109	0.122	0.000	0.008	0.061	-0.026	-0.316	0.048	0.000
Mean					0.068	-0.133	0.067				0.000	0.050	0.000	0.000	0.000	0.000	-0.063	-0.073	0.000
SD					1.120	1.221	1.326				1.000	1.065	0.000	1.000	1.000	1.000	1.093	1.128	0.000
z<-3					0	2	1				0	0	0	0	0	0	1	1	0
-3≤z<-2					0	3	0				0	4	0	1	1	0	2	2	0
-2<z≤3					0	1	3				2	0	0	2	2	0	0	1	0
z>3					1	0	2				0	1	0	0	0	1	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	
			-0.175	0.724	0.000							-0.698	-0.344	0.615	-0.358	0.010	-0.797	9441
			0.826	1.010	0.000				0.402	0.000	0.039	0.784	0.396	1.198	-3.141	0.899	1.646	9524
			-0.251	0.636	0.000				0.898	0.000	1.674	0.784	-0.998	0.345	-0.869	0.075	0.930	9736
			0.193	0.275	0.000				0.402	0.000	0.039	0.479	1.320	0.965	-0.358	1.203	0.930	9899
									0.402	0.000	0.039	-0.698	-1.911	-2.799	-0.358	0.838	4.000	9903
0	0	0	64	64	64	0	0	0	49	49	49	79	79	76	65	65	65	n
			-4.000	-4.000	0.000				-2.474	0.000	-2.554	-2.722	-4.000	-2.799	-3.141	-2.384	-0.797	Min
			4.000	2.068	0.000				1.982	0.000	1.674	4.000	4.000	2.621	4.000	4.000	4.000	Max
			-0.157	0.091	0.000				-0.148	0.000	0.039	0.141	-0.155	0.055	0.072	0.010	-0.797	Median
			0.063	-0.187	0.000				0.000	0.000	0.000	0.101	0.000	0.000	0.062	0.062	0.362	Mean
			1.308	1.296	0.000				1.000	0.000	1.000	1.172	1.177	1.000	1.109	1.109	1.489	SD
			1	3	0				0	0	0	0	1	0	1	0	0	Summa
			3	2	0				1	0	3	3	2	2	0	1	0	11
			2	1	0				0	0	0	3	3	1	1	2	1	30
			2	0	0				0	0	0	2	1	0	1	1	6	25
																		19

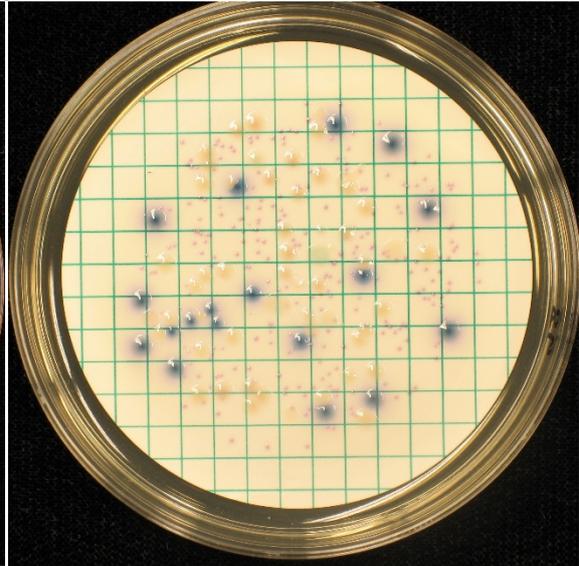
Sample A

m-Endo Agar LES, 37 °C



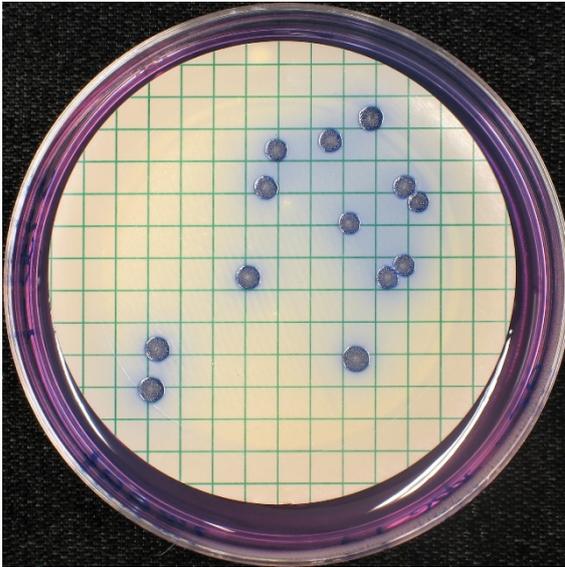
100 ml

Chromocult Coliform Agar, 37 °C



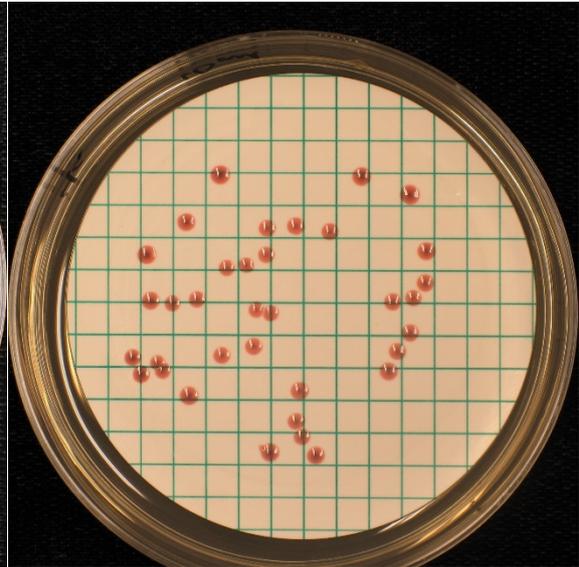
100 ml

m-FC Agar, 44 °C



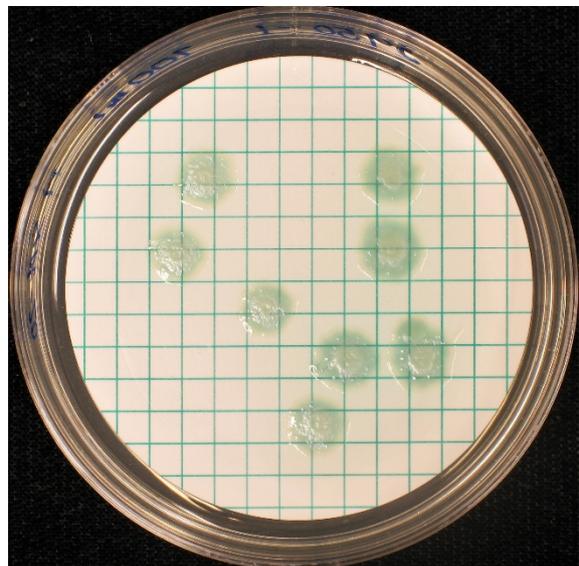
100 ml

m-Enterococcus Agar, 37 °C



10 ml, 2 days

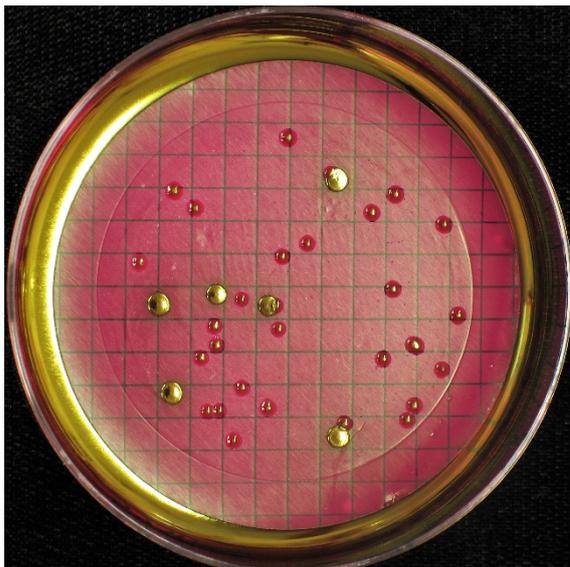
m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

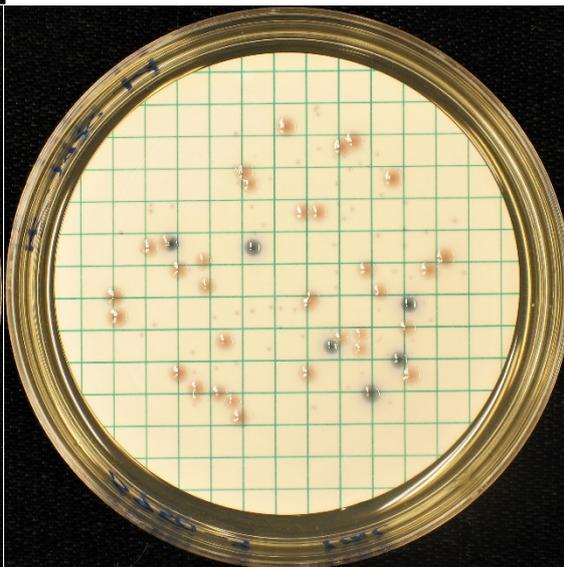
Sample B

m-Endo Agar LES, 37 °C



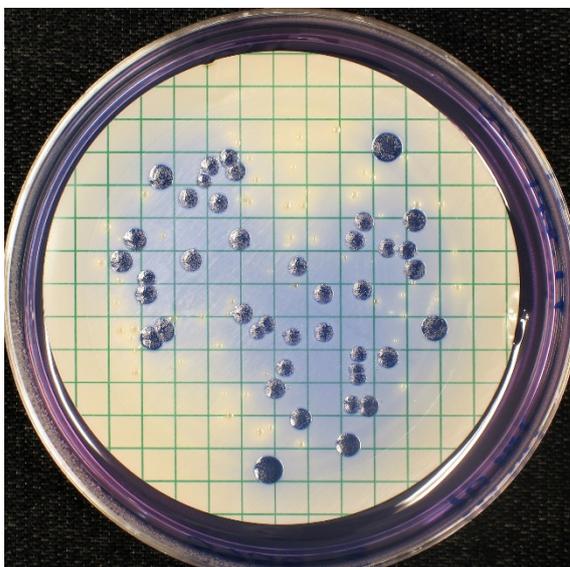
1 ml

Chromocult Coliform Agar, 37 °C



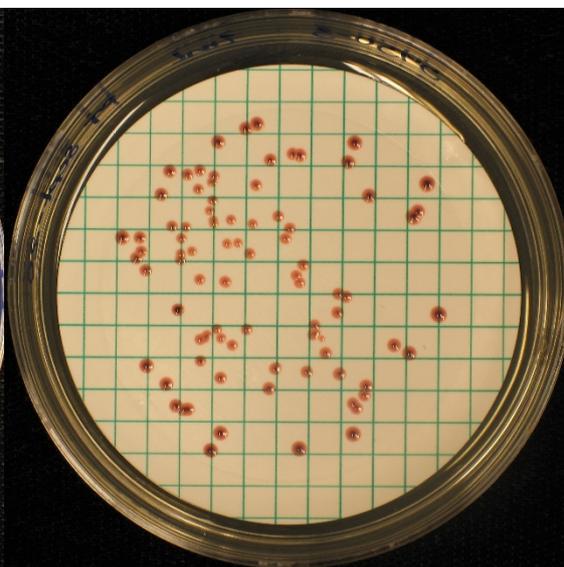
1 ml

m-FC Agar, 44 °C



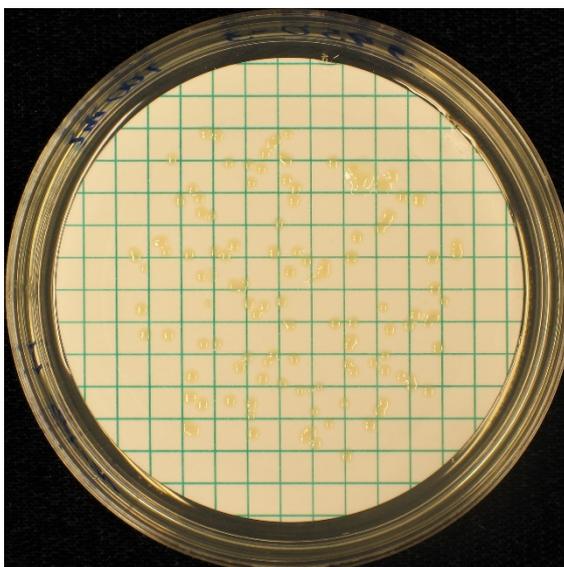
10 ml

m-Enterococcus Agar, 37 °C



1 ml, 2 days

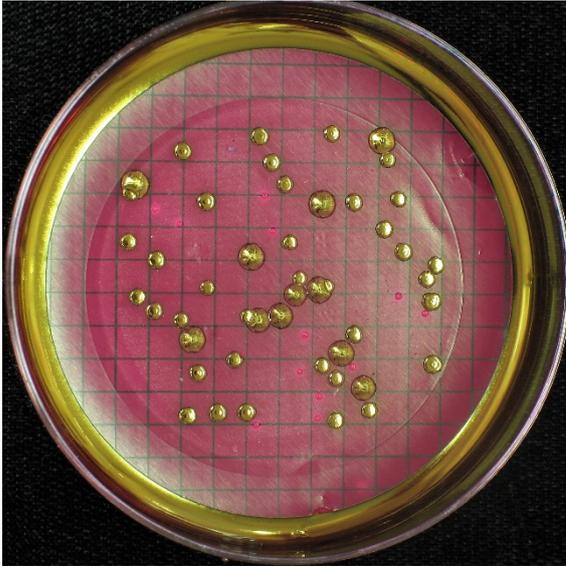
m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

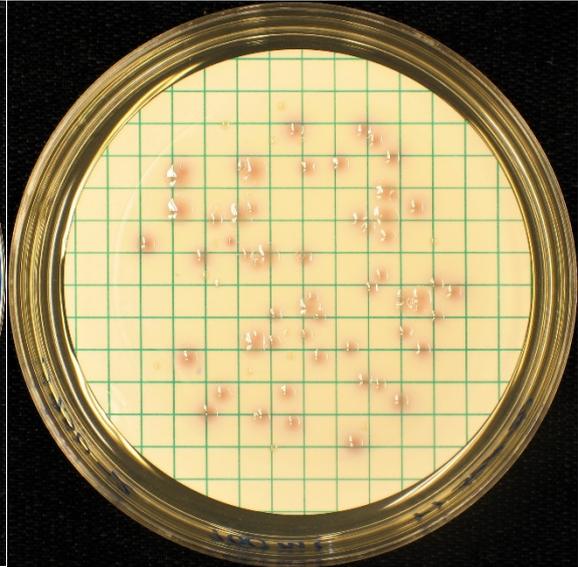
Sample C

m-Endo Agar LES, 37 °C



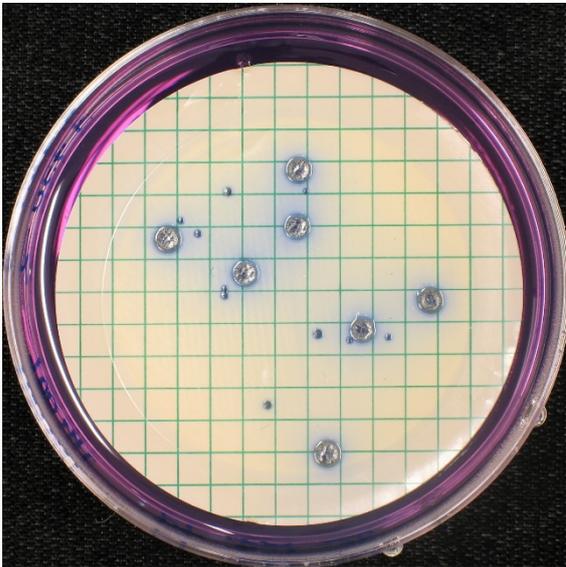
100 ml

Chromocult Coliform Agar, 37 °C



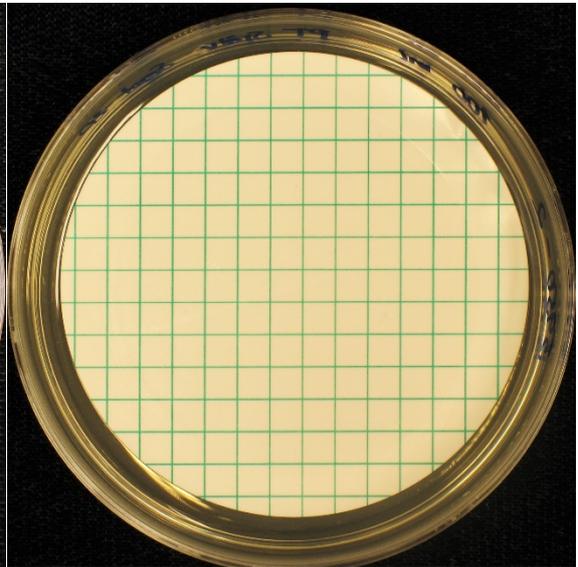
100 ml

m-FC Agar, 44 °C



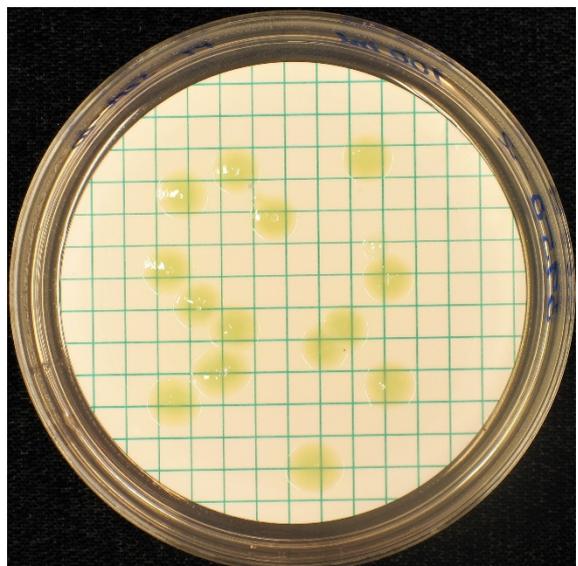
100 ml

m-Enterococcus Agar, 37 °C



100 ml, 2 days

m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

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

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

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

PT reports published 2020

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

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

Proficiency Testing – Food Microbiology, April 2020, 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>