

Drinking Water Microbiology

September 2014

by Tommy Šlapokas and Kirsi Mykkänen



Edition

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Editor in chief

Hans Lindmark, Head of Microbiology Division, National Food Agency

Responsible for the scheme

Tommy Šlapokas, Microbiologist, Microbiology Division, National Food Agency

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Proficiency testing
Drinking water Microbiology
September 2014



Parameters included

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

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

Intestinal enterococci with MF

Pseudomonas aeruginosa with MF

Culturable microorganisms (total count) 3 days incubation at 22 ± 2 °C

Culturable microorganisms (total count) 2 days incubation at 36 ± 2 °C

Tommy Šlapokas & Kirsi Mykkänen

Abbreviations and explanations




Microbiological media

LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (acc. to EN-ISO 9308-1:2000)
m-FC	m-FC Agar (acc. to SS 028167)
m-Ent	m-Enterococcus Agar (Slanetz & Bartley; according to EN ISO 8799-2:2000)
PACN	Pseudomonas Agar base with cetrimide and nalidixic acid (according to EN ISO 16266:2008)
YeA	Yeast extract Agar (acc. to EN ISO 6222:1999)
CCA	Chromocult Coliform Agar [®] (Merck; EN ISO 9308-1:2014)
Colilert	Colilert [®] Quanti-Tray [®] (IDEXX Inc.; EN ISO 9308-2:2014)

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

Tot 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

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General information on results evaluation

The histograms and calculation of outliers are described on page 31 under "Processing of numerical results" with further reference to the scheme protocol (1).

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

The method information gathered is sometimes difficult to interpret. Sometimes there is no consistency between the standard referred to and the information given 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.

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 measure of dispersion is not shown for groups with 4 or fewer results.

Results of the PT round September 2014

General outcome

Test items were sent to 110 laboratories, 36 in Sweden, 58 in other Nordic countries (Faeroe Islands and Åland included), 3 more from EU, 7 from the rest of Europe and 6 from countries outside Europe. Results were reported from 109 laboratories.

The percentages of false results and outliers are compiled in **table 1**. These deviating results are excluded in most calculations. This time the test material for the "mixtures" B and C originated from the same original mixture, here called B/C.

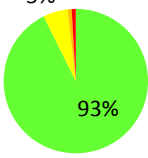
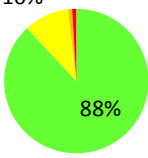
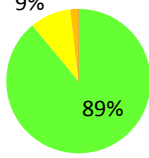
Microorganisms and parameters of analyses are also compiled in **table 1**. For the MF analyses the parameters *suspected* coliform bacteria and thermotolerant coliform bacteria, as well as *suspected* intestinal enterococci and *Pseudomonas aeruginosa* on primary media could be reported as well. The results from suspected colonies are only used for interpretations and discussions.

All reported results are compiled in **annex A**. Individual results for each laboratory are also shown on our website after logging in (www.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**.

Laboratory performance is described on page 25, after all analytical parameters, and illustrated by a box plot together with the number of deviating results for each laboratory.

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

Mixture	A			B			C		
Percentage of laboratories with   									
No. of evaluable results	617			618			617		
No. of deviating results *	11 (2 %)			16 (3 %)			14 (2 %)		
Microorganisms	<i>Escherichia coli (type 1)</i> <i>Escherichia coli (type 2)</i> <i>Serratia marcescens</i>			<i>Enterobacter cloacae</i> <i>Aeromonas hydrophila</i> <i>Enterococcus faecalis</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus warnerii</i>			<i>Enterobacter cloacae</i> <i>Aeromonas hydrophila</i> <i>Enterococcus faecalis</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus warnerii</i>		
	Analysis	Target	F%	X%	Target	F%	X%	Target	F%
Coliform bacteria (MF)	<i>E. coli (type 1)</i> <i>E. coli (type 2)</i> { <i>S. marcescens</i> }	1	0	<i>E. cloacae</i> [<i>A. hydrophila</i>]	1	6	<i>E. cloacae</i> [<i>A. hydrophila</i>]	1	5
Susp. thermotolerant coliform bact. (MF)	<i>E. coli (type 1)</i> <i>E. coli (type 2)</i>	–	–	<i>E. cloacae</i>	–	–	<i>E. cloacae</i>	–	–
<i>E. coli</i> (MF)	<i>E. coli (type 1)</i> <i>E. coli (type 2)</i>	0	0	[<i>E. cloacae</i>]	1	–	[<i>E. cloacae</i>]	1	–
Coliform bacteria (rapid method)	<i>E. coli (type 1)</i> <i>E. coli (type 2)</i> <i>S. marcescens</i>	2	2	<i>E. cloacae</i>	0	0	<i>E. cloacae</i>	0	3
<i>E. coli</i> (rapid meth.)	<i>E. coli (type 1)</i> <i>E. coli (type 2)</i>	0	2	–	0	–	–	0	–
Intestinal enterococci (MF)	–	1	–	<i>E. faecalis</i>	0	1	<i>E. faecalis</i>	0	1
<i>Pseudomonas aeruginosa</i> (MF)	–	2	–	<i>P. aeruginosa</i>	0	2	<i>P. aeruginosa</i>	0	3
Culturable microorganisms (total count), 3 days 22 °C	<i>S. marcescens</i> <i>E. coli (type 1)</i> <i>E. coli (type 2)</i>	0	3	<i>S. warnerii</i> <i>E. faecalis</i> <i>E. cloacae</i> <i>A. hydrophila</i> (<i>P. aeruginosa</i>)	0	1	<i>S. warnerii</i> <i>E. faecalis</i> <i>E. cloacae</i> <i>A. hydrophila</i> (<i>P. aeruginosa</i>)	0	1
Culturable microorganisms (total count), 2 days 36 °C	<i>S. marcescens</i> <i>E. coli (type 1)</i> <i>E. coli (type 2)</i>	0	2	<i>S. warnerii</i> <i>E. faecalis</i> <i>E. cloacae</i> <i>A. hydrophila</i> (<i>P. aeruginosa</i>)	0	7	<i>S. warnerii</i> <i>E. faecalis</i> <i>E. cloacae</i> <i>A. hydrophila</i> (<i>P. aeruginosa</i>)	0	2

* In total 23 of 109 laboratories (21%) reported at least one deviating result

– Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

[] The organism is false positive on the primary growth medium

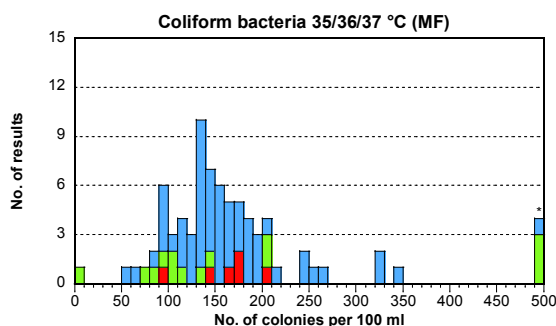
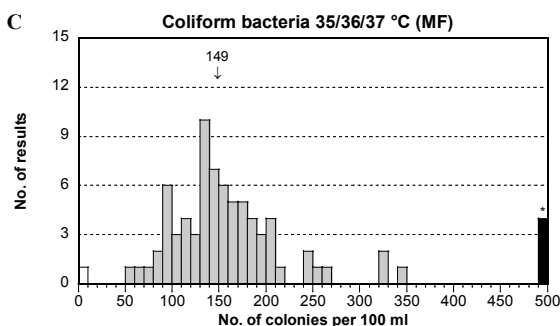
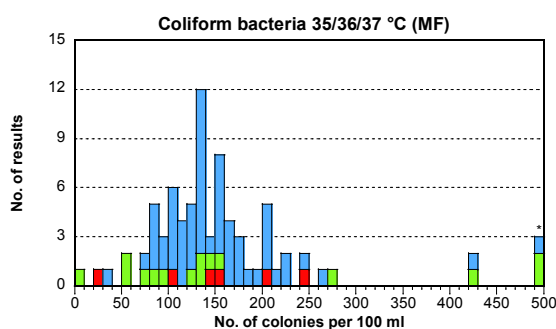
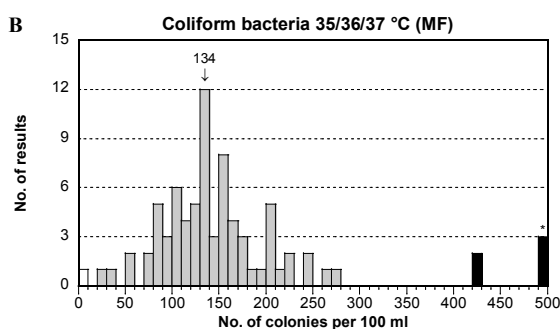
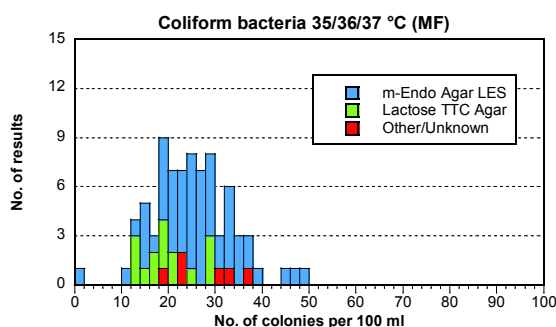
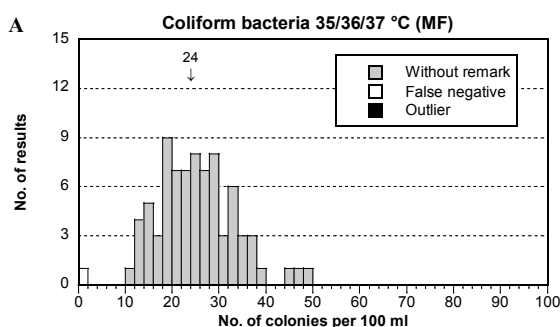
{ } The organism may give different results depending on method or definition used

Coliform bacteria (MF)

In two cases the primary medium reported was not the one prescribed in the standard referred to. There we have assumed the medium to be correct. The medium Endo Agar reported by some participants is here included in m-Endo Agar LES (LES).

From the table it is clear that LES was almost four times more frequently used than LTTC. There is an indication that LTTC gave a somewhat lower mean result compared to LES in all mixtures. However, the relative dispersion was equal for

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	79	78	24	16	1	0	0	73	134	20	1	0	5	73	149	18	1	0	4
m-Endo Agar LES	58	57	25	16	1	0	0	56	139	17	0	0	2	57	154	18	0	0	1
Lactose TTC Agar	15	15	19	16	0	0	0	11	111	26	1	0	3	10	120	18	1	0	3
Other/Unknown	6	6	27	14	0	0	0	6	132	33	0	0	0	6	156	12	0	0	0



LTTC and LES in 2 of the 3 mixtures. The category Other/Unknown medium does not show deviating results for any mixture.

Mixture A

- Only the two strains of *E. coli* grow with typical colonies, with a metallic sheen on LES and yellow on LTTC at 37 °C. The average recovery is well in accordance with that for *E. coli* with the rapid methods (see page 12).
- The strain of *S. marcescens* grows with atypical colonies, light red to pink on LES and greenish on LTTC. In a few cases these colonies might have been included as coliform bacteria.

Mixture B/C

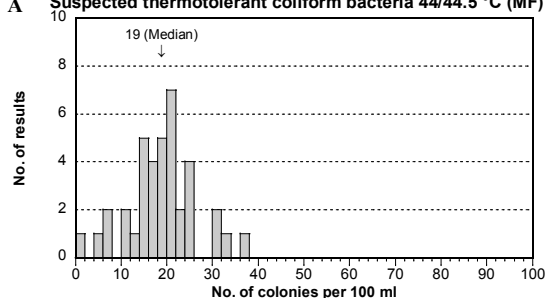
- The colonies of *E. cloacae* grow as typical suspected coliform bacteria on both LES and LTTC. Strains of *A. hydrophila* usually also grow with more or less typical colonies on these media. *A. hydrophila* is oxidase positive and the colonies can be excluded from coliform bacteria after confirmation by the oxidase test.
- The used *A. hydrophila* strain seems – at least occasionally – to have weaker metallic sheen than *E. cloacae* on LES and consequently more reddish colonies. This is evident from the photography of LES from mixture B/C in annex C. On LTTC it is more difficult to discern if the colonies are less typical than other strains. Yellow colour in the LTTC medium from individual colonies is difficult to discern, as the whole medium often turns yellow when many colonies are growing.
- The average number of coliform bacteria in this analysis is in good accordance with the number from the corresponding rapid method. This indicates that *A. hydrophila* has in principle always been correctly excluded by the MF method.

Suspected thermotolerant coliform bacteria (MF)

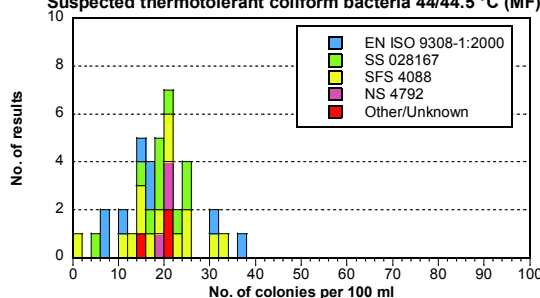
The two most used growth media were m-FC and LTTC. The incubation temperature was 44 or 44.5 °C. Here, results were separated based on the method standards most commonly used, to get a further division beyond the media. They are EN ISO 9308-1:2000 with LTTC and three standards with m-FC from the Nordic countries, namely SS 028167 from Sweden, SFS 4088 from Finland and NS 4792 from Norway. The methods were sometimes used slightly modified.

Standard, Method	Tot n	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
Total	38	38	19	–	–	–	38	13	–	–	–	38	9	–	–	–
EN ISO 9308-1	8	8	16	–	–	–	8	57	–	–	–	8	60	–	–	–
SS 028167	10	10	19	–	–	–	10	7	–	–	–	10	0	–	–	–
SFS 4088	14	14	20	–	–	–	14	52	–	–	–	14	74	–	–	–
NS 4792	3	3	20	–	–	–	3	0	–	–	–	3	0	–	–	–
Other/Unknown	3	3	20	–	–	–	3	0	–	–	–	3	0	–	–	–

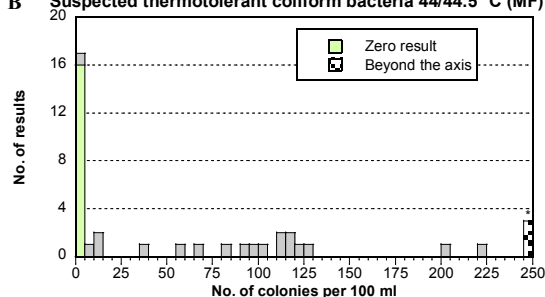
A Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



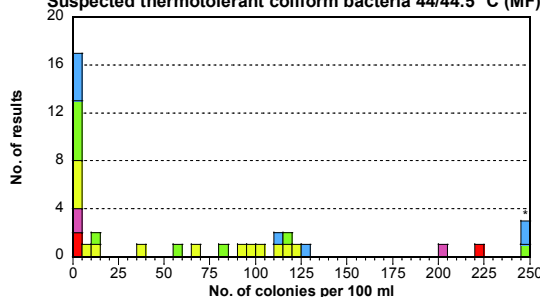
Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



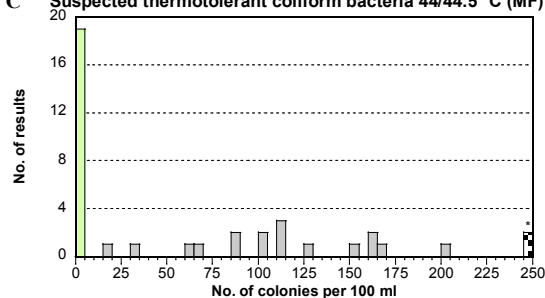
B Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



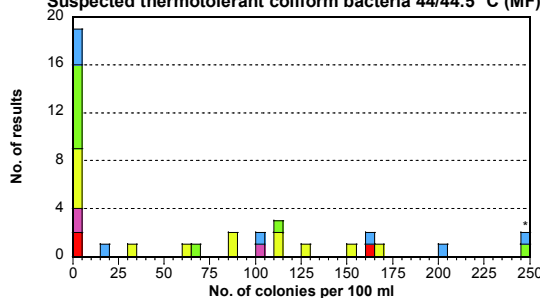
Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



C Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



The table shows the *medians* instead of mean values because no outliers have been identified. The reason is that *the analysis is not included in performance assessment*.

The Swedish standard states incubation at 44 °C but one laboratory reported 44.5 °C. The temperature 44 °C is also stated in EN ISO 9308-1:2000. All laboratories using Norwegian standard has this time incubated at 44.5 °C according to the standard, while all laboratories using Finnish standard has incubated at 44 °C, also according to the standard.

No general correlation between standard, and thus indirectly temperature, and colony recovery can be seen from the method histograms, not even for the mixtures B and C where the results are very scattered due to the absence of a typical thermotolerant coliform bacteria. Also at 44.5 °C with the Norwegian standard, NS 4792, have colonies been found in one laboratory.

Mixture A

- Colonies of the two strains of *E. coli* appear with blue colonies on m-FC at 44/44.5 °C. The corresponding colonies are orange-yellow on LTTC.
- The average result was somewhat lower on LTTC than on m-FC.

Mixture B/C

- The strain of *E. cloacae* seems, based on the results, sometimes to grow as a (suspected) thermotolerant coliform bacterium on both m-FC and LTTC. However, the results are very varying due to the fact that *E. cloacae* is not a typical thermotolerant coliform bacterium. The scattered results are seen also within the different methods used. Colonies appear also at the temperature 44.5 °C.
- A lot of zero results were obtained with each of the methods reported.

Escherichia coli (MF)

E. coli is quantified after confirmation of colonies that have grown either at 36±2 °C or at 44/44.5 °C. The primary growth media LTTC or LES are used at 36 °C and LTTC or m-FC at 44/44.5 °C. The results from the two temperatures are here shown in separate tables. The 44 results with unclear incubation temperature are not separately shown but are included only in the table "All results".

E. coli was present only in mixture A. Neither at 36±2 nor at 44/44.5 °C was a difference between methods seen, probably partly due to the very few results from several media.

In each of mixture B and C was only one false positive result present. These two results were reported by the same laboratory.

Mixture A

- Two typical *E. coli* strains were included in the mixture. To confirm the presence of *E. coli*, test of indole production or β -glucuronidase activity has to be done from all media, incubated either at 36±2 or at 44/44.5 °C No deviating results were present.

All results

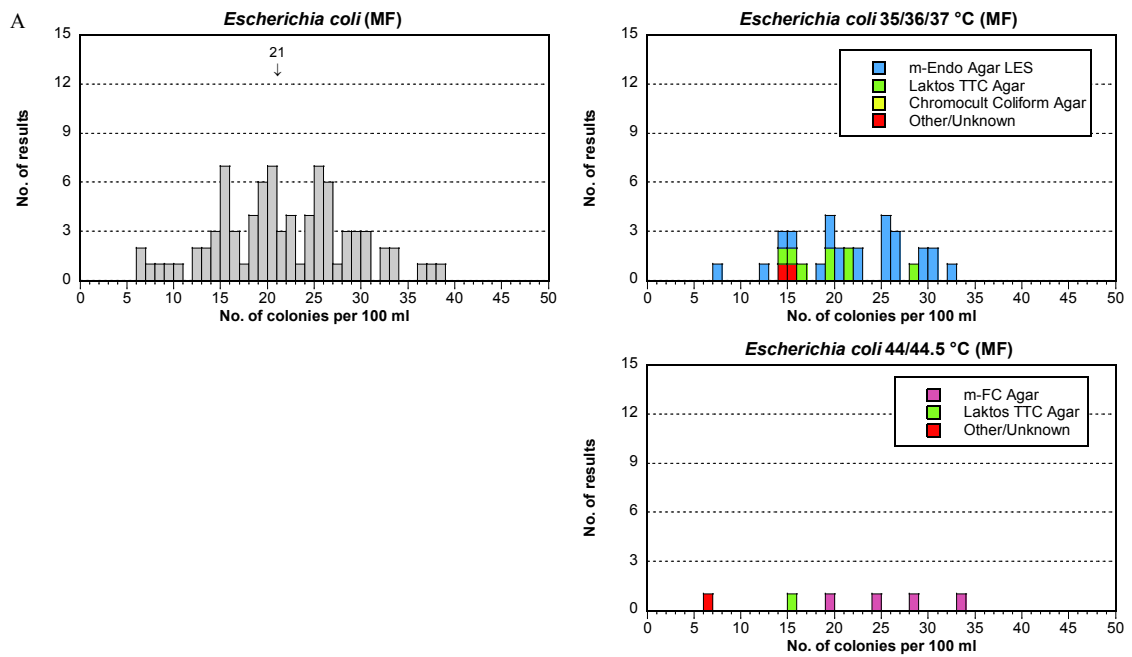
Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	83	83	21	18	0	0	0	82	0	-	1	-	-	82	0	-	1	-	-

From 36±2 °C

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	33	33	21	15	0	0	0	33	0	-	0	-	-	33	0	-	0	-	-
m-Endo Agar LES	23	23	22	16	0	0	0	23	0	-	0	-	-	23	0	-	0	-	-
Lactose TTC Agar	8	8	19	11	0	0	0	8	0	-	0	-	-	8	0	-	0	-	-
Chromocult C Agar	0	0	-	-	-	-	-	0	0	-	-	-	-	0	0	-	-	-	-
Other/Unknown	2	2	14	-	0	0	0	2	0	-	0	-	-	2	0	-	0	-	-

From 44/44.5 °C

Medium/Standard	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	6	6	20	27	0	0	0	6	0	-	0	-	-	6	0	-	0	-	-
<i>Medium</i>																			
m-FC Agar	4	4	26	-	0	0	0	4	0	-	0	-	-	4	0	-	0	-	-
Lactose TTC Agar	1	1	15	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-
Other/Unknown	1	1	6	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-
<i>Standard</i>																			
EN ISO 9308-1	2	2	10	-	0	0	0	2	0	-	0	-	-	2	0	-	0	-	-
SS 028167	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
SFS 4088	2	2	30	-	0	0	0	2	0	-	0	-	-	2	0	-	0	-	-
NS 4792	1	1	19	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-
Other/Unknown	1	1	24	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-



- Both with incubation at 36 ± 2 and $44/44.5$ °C there is a tendency that LTTC according to the standard EN ISO 9308-1:2000 yields lower average result than LES or m-FC according to the Nordic standards.

Mixture B/C

- No *E. coli* was included in the mixture. However, the strain of *E. cloacae* will appear at 36 ± 2 and more or less also at $44/44.5$ °C. The strain will be removed as presumptive *E. coli* due to the lack of indole production and β -glucuronidase activity.

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

The rapid method used for both these parameters was almost exclusively Colilert® Quanti-Tray® from the manufacturer IDEXX Inc. Two laboratories have not used a rapid method but the classical multiple tube method with MPN quantification (Standard Methods 9221B; 5) for coliform bacteria. Out of the about 60 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 wells (a few of which, probably incorrectly, have reported 96 wells). The laboratories often analysed both diluted and undiluted samples. The only laboratory in the category Other/Unknown stated the use of "Colilert 24 hours".

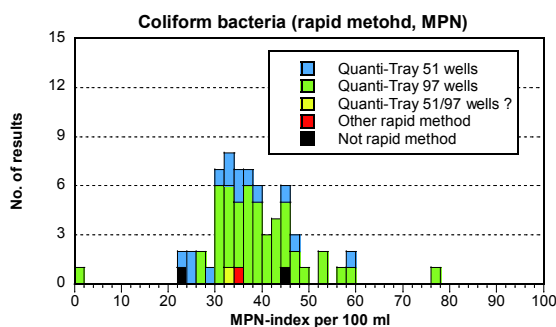
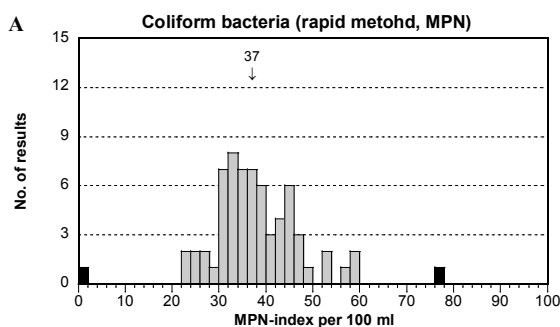
For coliform bacteria in all mixtures there was a tendency that the trays with 51 wells give somewhat lower average recovery than trays with 97 wells. This could not be seen for *E. coli* in mixture A. Five outlying results were seen, with 2 each from 2 laboratories.

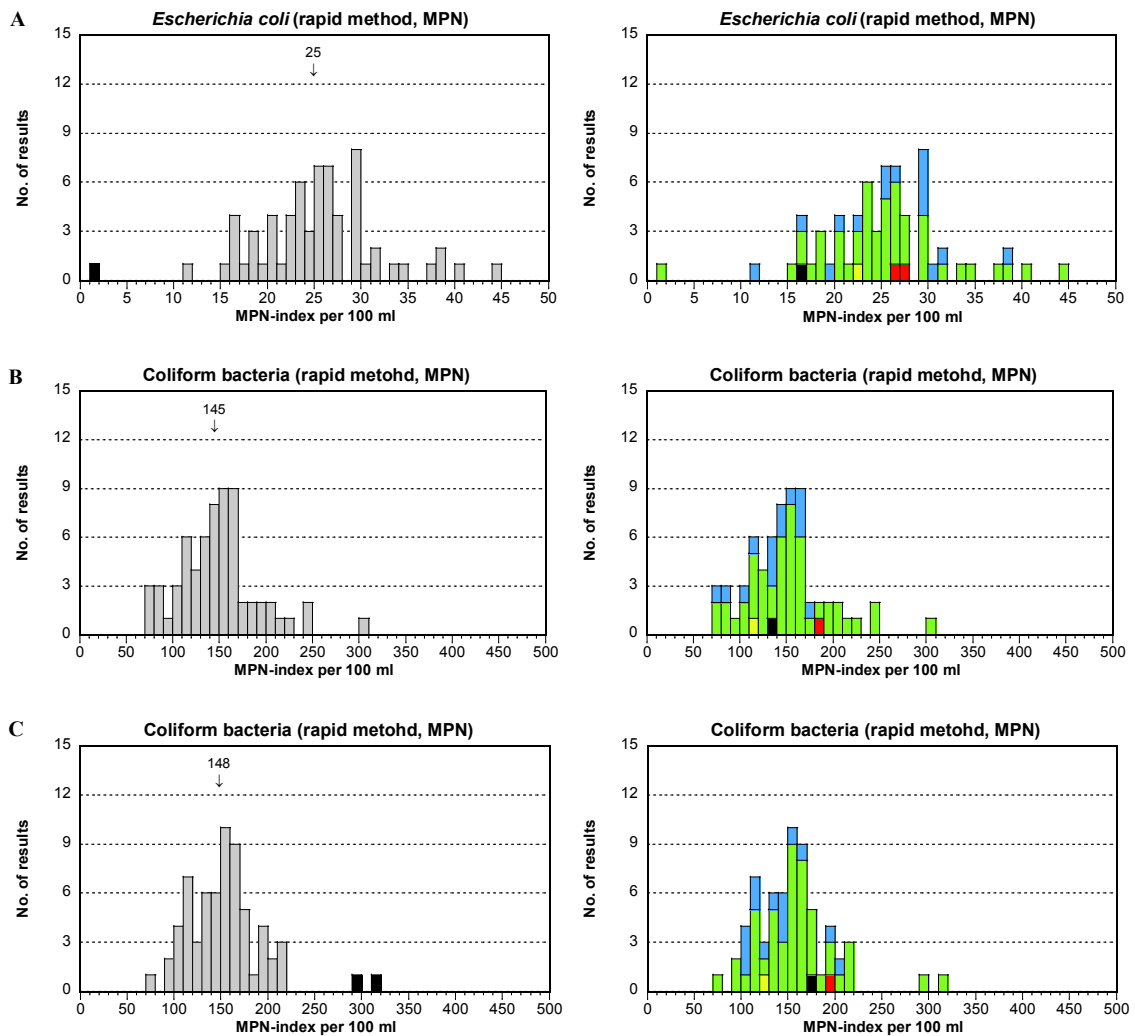
Coliform bacteria, Rapid method with MPN

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	64	62	37	10	0	1	1	64	145	14	0	0	0	62	147	11	0	0	2
Colilert Quanti-51	14	14	34	14	0	0	0	14	133	12	0	0	0	14	137	10	0	0	0
Colilert Quanti-97	48	46	38	9	0	1	1	48	149	15	0	0	0	46	150	11	0	0	2
Colilert Quanti-?	1	1	33	-	0	0	0	1	111	-	0	0	0	1	122	-	0	0	0
Other/Unknown	1	1	35	-	0	0	0	1	180	-	0	0	0	1	190	-	0	0	0
Not rapid method	2	2	33	-	0	0	0	2	130	-	0	0	0	1	170	-	0	0	0

E. coli, Rapid method with MPN

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	65	64	25	12	0	1	0	65	0	-	0	-	-	65	0	-	0	-	-
Colilert Quanti-51	15	15	25	14	0	0	0	15	0	-	0	-	-	15	0	-	0	-	-
Colilert Quanti-97	47	46	25	12	0	1	0	47	0	-	0	-	-	47	0	-	0	-	-
Colilert Quanti-?	1	0	22	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-
Other/Unknown	2	2	26	-	0	0	0	2	0	-	0	-	-	2	0	-	0	-	-
Not rapid method	1	1	16	-	0	0	0	1	0	-	0	-	-	1	0	-	0	-	-





Mixture A

- The two strains of *E. coli* together with *S. marcescens* grow and possess β -galactosidase. They are thus detected as coliform bacteria by methods based on the activity of this enzyme (ONPG positive), e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate.
- The strain of *S. marcescens* is here detected as a coliform bacterium but is an atypical coliform bacterium when using MF methods based on fermentation of lactose and detection of gas or aldehyde production. Accordingly, the average result is higher here compared to by the MF methods reported.
- Both strains of *E. coli* possess the enzyme β -glucuronidase and is also detected as *E. coli*.

Mixture B/C

- In these mixtures *E. cloacae* was the only coliform bacterium. It possesses β -galactosidase but not β -glucuronidase and is detected as a coliform bacterium but not as an *E. coli*. The average results were about the same as for the MF-methods.

Intestinal enterococci (MF)

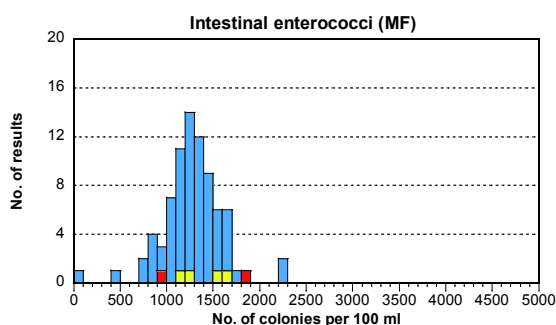
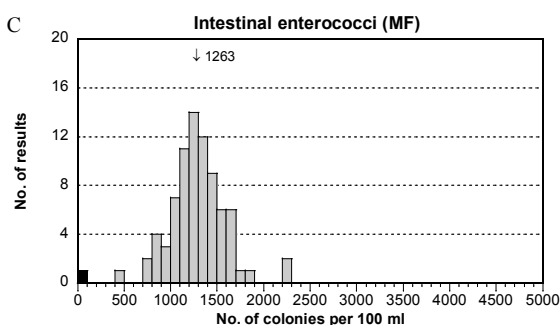
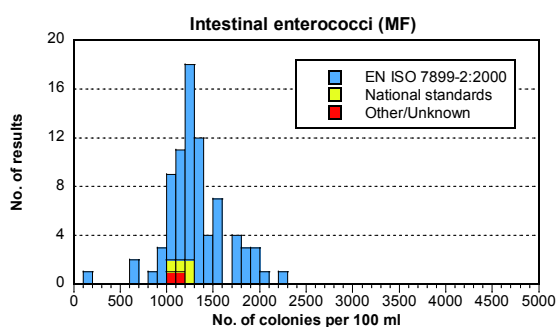
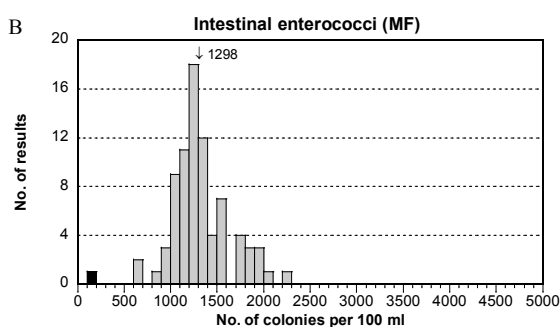
The method EN ISO 7899-2:2000 with m-Enterococcus Agar was almost always used. In only 6 cases another method reference has been stated. Also in these cases the medium m-Enterococcus Agar has been used as primary medium. Sometimes the medium is in the comments referred to as Slanetz & Bartley Agar, which is the same medium. Such comments are sometimes also found when EN ISO 7899-2:2000 is given as reference. In on laboratory "Enterolert" has been used, in spite of not being an MF method.

The reported temperature for incubation was always 36 ± 2 °C, and confirmation was in 73% of the cases performed with Bile-esculine-azide agar (BEA Agar) as is stated in EN ISO 7899-2:2000. Confirmation was in 15% performed on Bile-esculine agar (without azide; BE Agar). It is difficult to know if this difference is real or is due to terminology mixing-up. The temperature for confirmation was in 91% of the laboratories 44 °C and in 6% 44.5 °C.

Discussions about method differences are meaningless since the method for presumptive intestinal enterococci does not differ for the vast majority of the results

Intestinal enterococci MF

Standard	Tot n	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
Total	80	78	0	-	1	-	79	1298	11	0	1	0	79	1263	12	0	1	0
EN ISO 7899-2	74	73	0	-	0	-	73	1312	12	0	1	0	73	1255	12	0	1	0
National standards	4	3	0	-	1	-	4	1165	-	0	0	0	4	1367	-	0	0	0
Other/Unknown	2	2	0	-	0	-	2	1073	-	0	0	0	2	1359	-	0	0	0



reported. There was no difference in the confirmation outcome in relation to BEA Agar or BE Agar.

Mixture A

- No intestinal enterococcus strain was included but one false positive result was reported.

Mixture B/C

- A typical strain of *E. faecalis* was present in the mixture. The dispersion of the results was good.
- One low outlier was reported from each of two laboratories in the B and C part of the mixture.

Pseudomonas aeruginosa (MF)

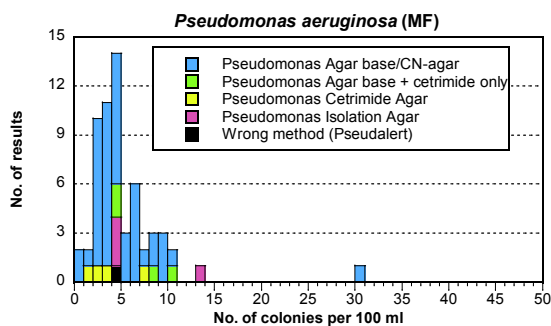
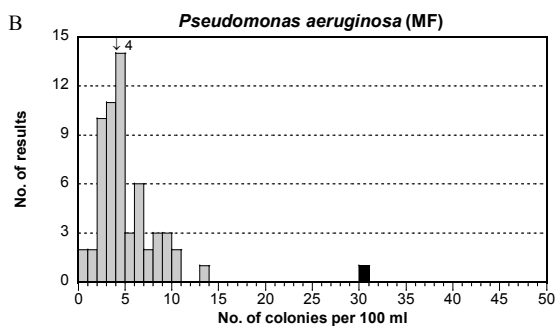
The method EN ISO 16266:2008 – with or without modification – was used by 56 out of the 60 laboratories reporting results for this analysis. Some of the laboratories have reported the method by reference to the identical, since long time withdrawn, CEN standard EN 12780:2002, with or without modification. Incubation was always done at 36±2 °C. Except in 4 cases where Pseudomonas Isolation agar was reported, and in one case where "Pseudalert" (Idexx Inc.) was used, the laboratories used "Pseudomonas Agar base" or "Pseudomonas Cetrимide Agar" with cetrимide and/or nalidixic acid (C/N-supplement) added. Confirmation tests are performed when needed according to the standards (atypical colonies). Modifications in the methods refer probably mainly to these confirmation tests.

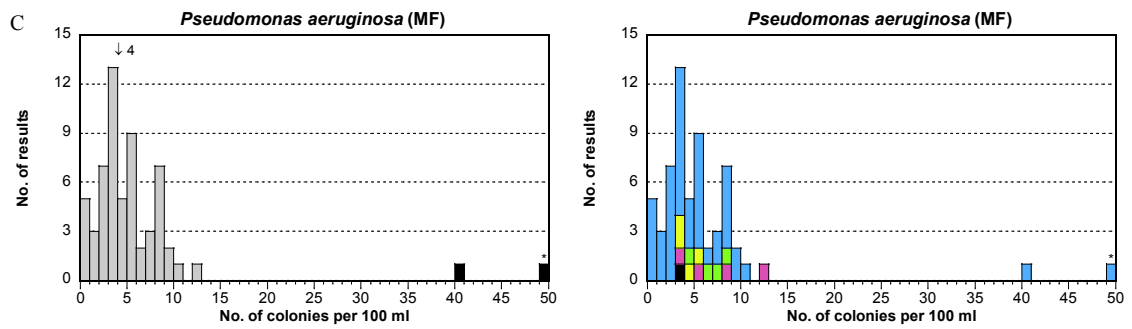
The base method and the primary cultivation medium for *P. aeruginosa* is the same for the majority of the results. Yet, the method differentiation is this time done in relation to base medium reported. The various supplements used, such as cetrимide (C) or nalidixic acid (N), are to some extent correlated to the medium stated. The laboratories that have reported Pseudomonas Isolation Agar have in 3 out of 4 cases used both cetrимide and nalidixic acid, and are in all cases referring to the standards EN ISO 16266:2008 or EN 12780:2002.

Because the numbers of results are very few in all but one group, it is impossible to tell if there are any differences in relation to base media. Generally, the results from media groups with few values seem scattered among those from Pseudomonas Agar base, C+N.

Pseudomonas aeruginosa MF

Medium used	Tot n	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
Total	60	59	0	–	1	–	59	4	32	0	0	1	58	4	41	0	0	2
Ps. Agar base, C+N	47	46	0	–	1	–	46	4	32	0	0	1	45	3	46	0	0	2
Ps. Agar base, C	4	4	0	–	0	–	4	6	–	0	0	0	4	6	–	0	0	0
Ps. Cetrимide Agar	4	4	0	–	0	–	4	3	–	0	0	0	4	4	–	0	0	0
Ps. Isolation Agar	4	4	0	–	0	–	4	6	–	0	0	0	4	7	–	0	0	0
Wrong method	1	1	0	–	0	–	1	4	–	0	0	0	1	3	–	0	0	0





Mixture A

- There was no *P. aeruginosa* in the mixture. One false positive result was reported.

Mixture B/C

- One strain of *P. aeruginosa* was included in the mixture but in low number. The implication is that also zero results are regarded as correct. The strain is typical with blue green pigmentation and fluorescence on the PACN medium.
- The distribution of the results was good despite the low average. Three laboratories reported each one high outlier.

Culturable microorganisms 22 °C, 3 days

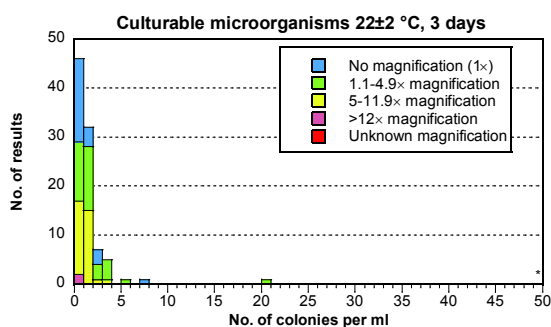
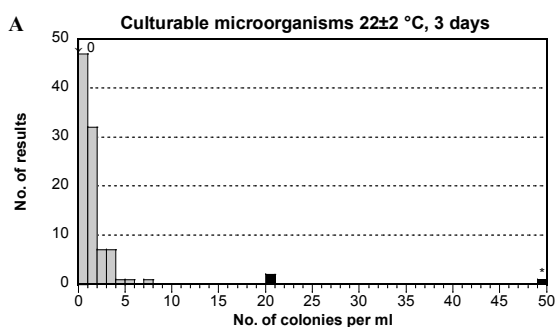
Only six of the 100 participating laboratories reported a method different from EN ISO 6222:1999. Ten laboratories used Plate Count Agar, of which 5 together with EN ISO 6222:1999. The other 5 used national standards or "Standard methods" (5). Two laboratories used Nutrient Agar out of which one used spread plating together with EN ISO 6222:1999 and the other membrane filtration and "Nutrient pads". Five more laboratories reported spread plating, most often in combination with EN ISO 6222:1999.

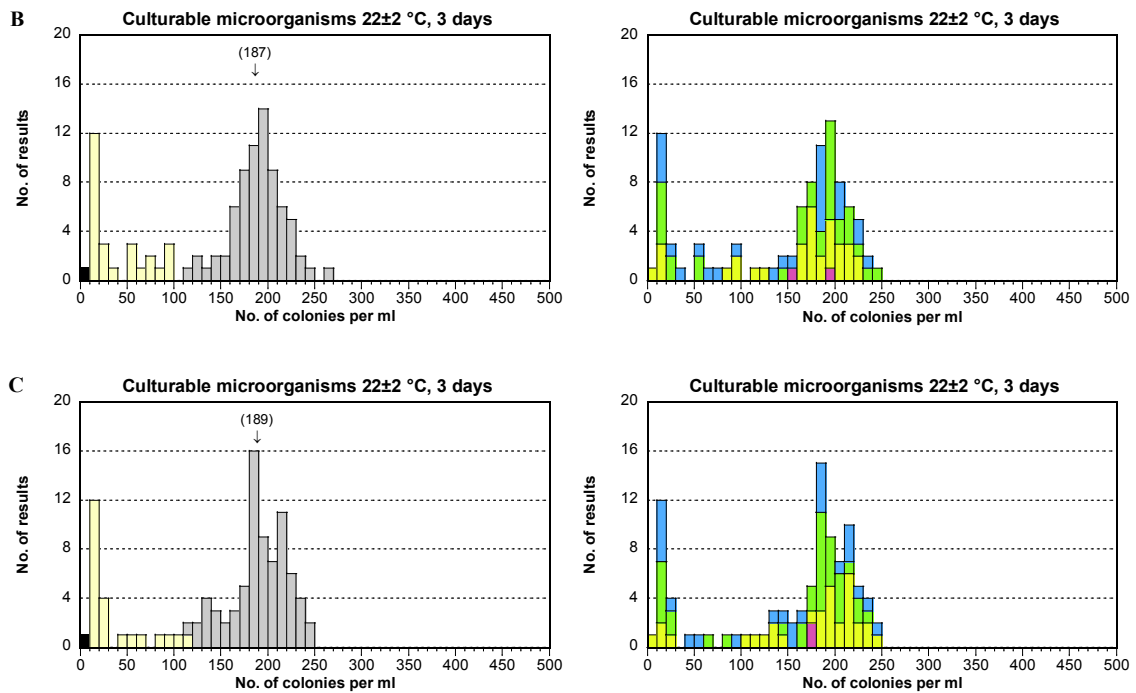
Only comparisons of method variants in connection to EN ISO 6222:1999 are shown and discussed. Results are grouped for culture media and magnification for reading.

For mixture A there are too low group averages to state any differences. For "mixtures" B and C relatively more low results have been obtained with no and low magnification (<5×) than with higher magnification, resulting in a lower average the lower the magnification is. However, some low results were present with higher magnification as well, indicated by comparison with the averages within brackets in the histograms (187 and 189 cfu/ml). They are the total ones obtained if only results ≥119 cfu/ml would have been considered as acceptable. Here most of the lower results have been accepted, represented by light yellow histogram bars (see below).

22±2 °C, 3 days

Group of results	Tot n	A					B					C							
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >			
Total, all results	100	96	0	108	0	0	3	98	134	33	0	1	0	98	140	32	0	1	0
<i>EN ISO 6222</i>	94	92	0	109	0	0	0	92	132	33	0	1	0	92	138	33	0	1	0
<i>Medium</i>																			
Yeast extract Agar	86	84	0	110	0	0	1	84	130	34	0	1	0	84	136	34	0	1	0
Plate Count Agar	5	5	1	109	0	0	0	5	135	36	0	0	0	5	138	33	0	0	0
Other/Unknown	3	3	1	-	0	0	0	3	188	-	0	0	0	3	207	-	0	0	0
<i>Magnification</i>																			
None	26	25	0	164	0	0	0	26	113	39	0	0	0	25	114	41	0	0	0
1,1-4,9×	34	33	1	85	0	0	1	33	130	37	0	0	0	34	133	35	0	0	0
5-11,9×	32	32	0	99	0	0	0	31	150	25	0	1	0	31	164	25	0	1	0
> 12×	2	2	0	-	0	0	0	2	169	-	0	0	0	2	172	-	0	0	0
Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<i>Other method</i>	6	4	2	-	0	0	2	6	164	21	0	0	0	6	162	15	0	0	0





Mixture A

- Colonies of all the three coliform bacteria can appear as culturable microorganisms but in very low numbers, resulting in a very low mean value.
- Three high outliers were present.

Mixture B/C

- The colonies consist almost entirely of *S. warnerii*. All other strains will also grow but in considerably lower number.
- The results show two evident peaks in the histograms. The first peak implies that colonies of *S. warnerii* have not been included. Thus, the colonies of that strain must have been so small that they were almost impossible to discern with the magnification used. Low results are in many cases also correlated with no or low magnification when reading the plates.
- The strain of *S. warnerii* has not behaved as expected. When the National Food Agency checked the vials, first for concentration and then homogeneity before dispatch of the test items, the results were homogenous and in the higher end of the results distribution. The means were 215 and 226 cfu/ml in the mixtures and no comments were given about small colonies, difficult to count. During the preliminary evaluation of the results it was seen that 20–25% of the participant results were lower than the rest of them in both mixtures B and C. This is also clear from the histograms above. Some additional analyses were then performed at the National Food Agency. The colonies of *S. warnerii* were this time very small, even sometimes hardly impossible to discern and count with confidence when magnification $> 10\times$ was used. Only 15-25 colonies were possible to count with any confidence. However, it was possible to see a lot of more very small particles, probably developing into colonies, which were difficult to count. The

reason for these small colonies this time is not obvious. Based on these findings it seems very plausible that the low results from one fourth of the laboratories are caused by such small, undeveloped colonies that hardly are possible to count even with magnification.

- As a consequence of the situation with the sometimes very small colonies of *S. warnerii*, almost impossible to count, the low results in the leftmost part of the histogram are not judged as erroneous. Only the two results <3 cfu/ml, caused by taking the common logarithm of the results obtained, are here judged as outliers. Because the averages and dispersions of the results not are the same for the groups of results, **no z-scores are calculated for culturable microorganisms 22 °C, 3 days in the "mixtures" B and C.**

Culturable microorganisms 36 °C, 2 days

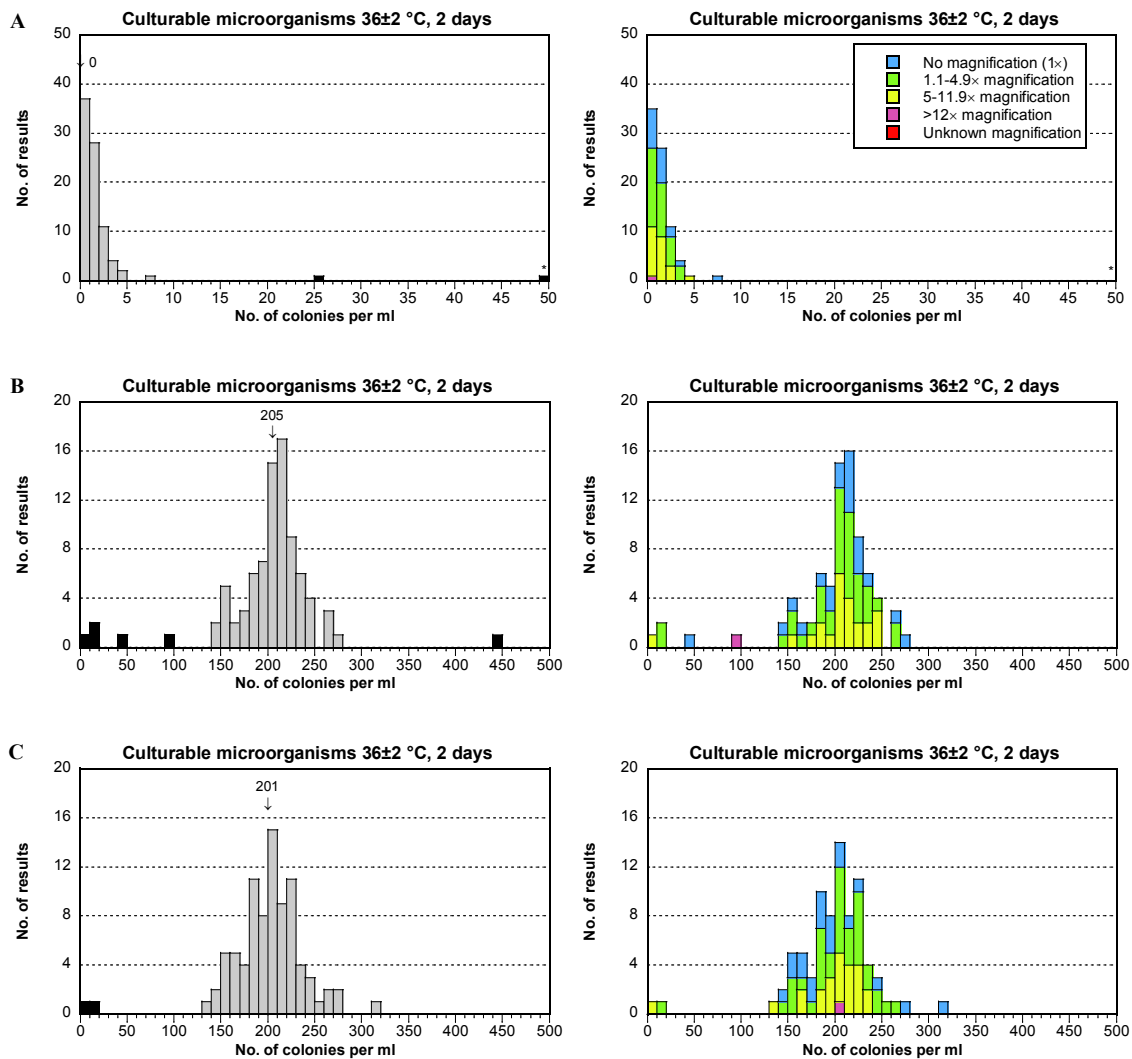
Only 6 out of 86 laboratories reported a method other than EN ISO 6222:1999. One high outlier was reported from those 6 laboratories in each of the two mixtures.

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

Neither for medium nor for magnification can differences be seen for the method variants in the mixtures,

36±2 °C, 3 days

Group of results	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	86	83	0	98	0	0	2	80	205	7	0	5	1	84	201	8	0	2	0
EN ISO 6222	80	79	0	98	0	0	1	75	207	7	0	5	0	78	200	8	0	2	0
<i>Medium</i>																			
Yeast extract Agar	72	72	0	96	0	0	0	69	208	7	0	3	0	70	201	8	0	2	0
Plate Count Agar	6	5	0	137	0	0	1	5	191	7	0	1	0	6	189	8	0	0	0
Other/Unknown	2	2	1	-	0	0	0	1	220	-	0	1	0	2	195	-	0	0	0
<i>Magnification</i>																			
None	20	19	1	102	0	0	1	19	206	8	0	1	0	20	194	10	0	0	0
1,1-4,9×	36	36	0	96	0	0	0	34	206	7	0	2	0	35	202	7	0	1	0
5-11,9×	23	23	0	96	0	0	0	22	209	5	0	1	0	22	201	6	0	1	0
> 12×	1	1	0	-	0	0	0	0	-	-	0	1	0	1	200	-	0	0	0
Unknown	0	0	-	-	-	-	-	0	-	-	0	0	0	0	-	-	-	-	-
Other method	6	4	1	-	0	0	1	5	186	6	0	0	1	6	217	9	0	0	0



Mixture A

- All the coliform bacteria in the mixture grow at 36±2 °C and contribute to the culturable microorganisms but in low numbers as in the analyses at 22 °C.
- Two high outliers were present.

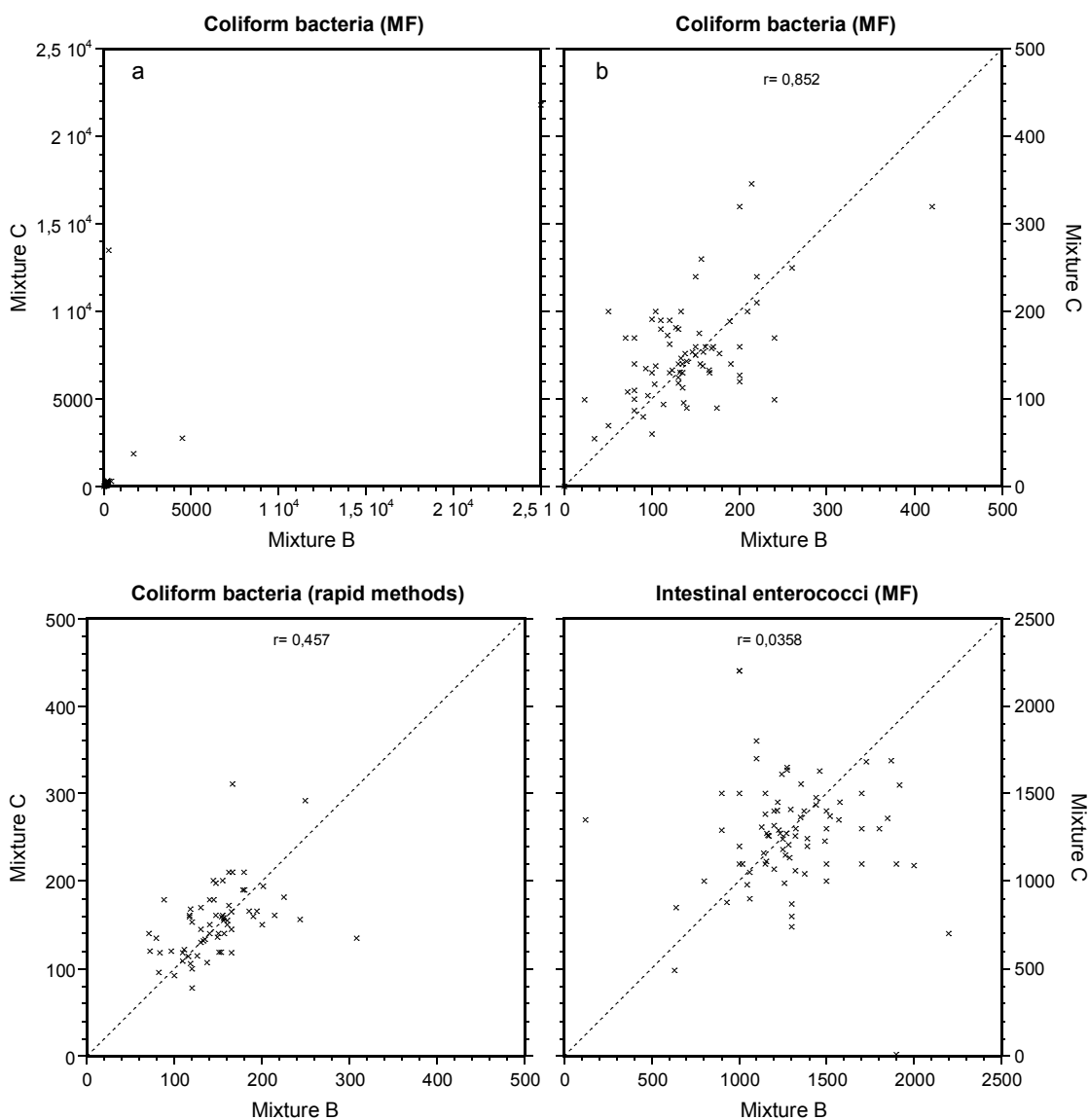
Mixture B/C

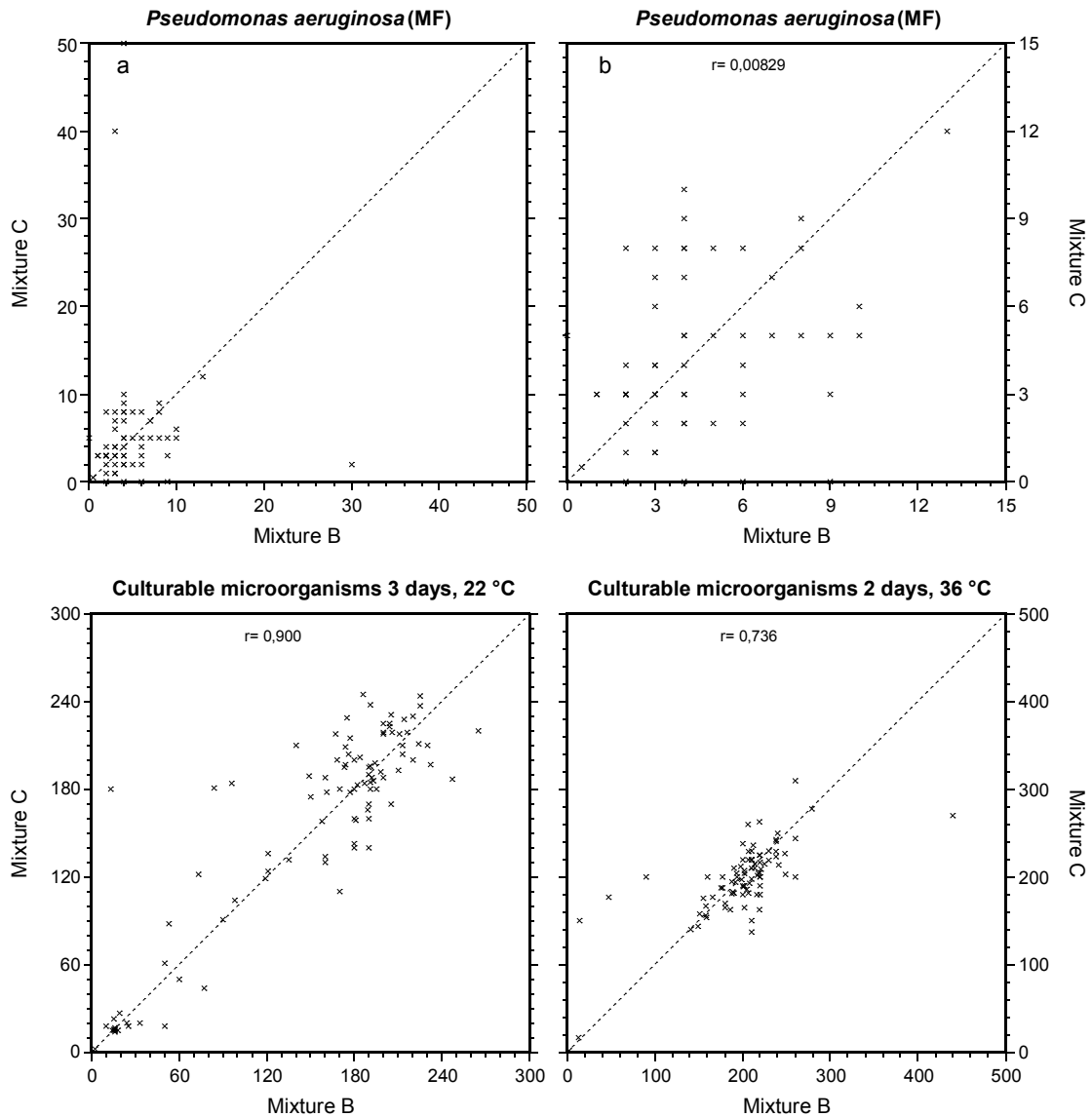
- The colonies are to a large extent composed of *S. warnerii*. All other strains also grow but in much lower numbers, as in the analysis at 22 °C.
- No apparent problem to find and count the colonies of *S. warnerii* was seen at this temperature. No tails with low results were neither seen in the mixtures B and C at this higher incubation temperature. Thus, the average results are higher.

Comparison of results from the “mixtures” B and C

Mixture B/C was analysed as samples belonging to mixture B and mixture C. In order to get an idea about if systematic differences between laboratories exist, or if the results may be randomly obtained, the result from mixture C is plotted against that from mixture B for each laboratory. The points obtained in this way are presented in the shape of an XY-plot (Youden-plot) for each analysis. Laboratories that have reported results from one mixture only are of course not included in the plots. For each relevant parameter one plot is presented with all points visible, and

XY plot The results from mixture C coupled to the results from mixture B, where r is the correlation coefficient; where there are two diagrams, the first shows all results and the second an enlarged picture with high outliers removed. The colony numbers are either stated as cfu/100 ml or cfu/ml depending on analysis.





where it is appropriate to get a better idea of the outcome, a second plot is shown where high deviating results are beyond the axis limits.

The better homogeneity in the mixture and the less random variation there is in the analysis, the more gathered the points are around a line going through the origin and where the slope is 1 (the 1:1 line in the plots). Where there are obvious systematic differences between laboratories the values are clearly spread along the line, while they in cases of non-systematic differences are randomly spread around the mean value in a more or less clear circular cluster.

The coefficient of correlation (r) is stated in one plot for each analysis. The value of r is 1.000 if the correlation is total (all values on the line) and 0 when there is no relation.

In all analyses there are individual points where the value is high or low on one axis, while it is about average on the other. Such high values are seen in the first plot for each analysis. The cause to these high results often stays unclear but indicates that something has gone wrong.

Systematic correlations are more or less obvious for the analyses of coliform bacteria (MF) and culturable microorganisms, both when looking on the point scatter and the relatively high values of r (0.74–0.90). The correlation might be due to varying skills of using an analytical method or because different method variants have been used. These variants can be different media, different incubation temperature or perhaps more plausible different confirmation routines or different magnification when reading the plates. However, no obvious correlations to results have been seen when looking at different method variants. Tendencies with lower results for some method variants are seen only for the membrane filter (MF) method and the rapid method for coliform bacteria (see the respective section).

The results for coliform bacteria (MF) were in average lower by use of LTTC compared to LES, but this explains only partly the correlation between the "mixtures". In both plot a and plot b for coliform bacteria (MF) it is clear that some laboratories have obtained high results for both mixtures. This indicates some kind of contamination or other systematic errors such as dilution mistakes or that the results are given for wrong volume unit.

For the analysis of coliform bacteria with rapid methods there are individual results far away from the main scatter of the results. The reason is not evident as it sometimes only one result in a pair of results that deviates. The overall somewhat ellipsoid appearance of the results scatter is at least partly caused by the difference due to choice of method.

Also for the intestinal enterococci are individual results far away from the main scatter. Since the rest of the results scatter not is clearly ellipsoid, those results seem to be randomly obtained. For *P. aeruginosa* the average results were very low. No systematic tendency seems to exist there, which also is apparent from the very low value of the correlation coefficient ($r=0.008$).

In the analysis of culturable microorganisms at 36 ± 2 °C there are also some deviating low and high results in mixture B. When these results are excluded a somewhat ellipsoid scatter is visible. Some kind of systematic variation thus seems to be present in this analysis. The cause of it is not clear from the method information gathered. Plate reading magnification was expected to be probable explanation.

The strong correlation ($r=0.90$) for culturable microorganisms at 22 °C is clearly related to the fact that $\frac{1}{4}$ of the laboratories reported very low results for both mixture B and C (see the discussion for that parameter). Without these low results the remaining results scatter is not typical ellipsoid but seem more randomly distributed. Of course, the appearance is affected by where the limit is drawn for which results to include.

Outcome of the results and laboratory assessment

General information about reported results

The distributions of results for the respective analysis are shown in 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 to summarize its performance. These values are highlighted with bold text and colour background in annex A, where all reported results are compiled. The limit values for lowest and highest accepted results are given for each analyse 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 assessment is basically a clear indication of the numbers of false results and outliers.

Generally, the laboratories that did not report their results in due time, have to compare their results themselves with all other laboratory's by looking in tables, figures and annex A.

Mixed up results and other practical errors

When whole samples seem to have been mixed up, the corresponding sample numbers are hatched in annex A. In this round no laboratory seems to have mixed up individual results or vials. One laboratory has erroneously reported results with common logarithms, leading to several low outliers. Furthermore, a number of laboratories have several deviating results that are somewhat dependent. No laboratory seems to have calculated the results for another volume than asked for.

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

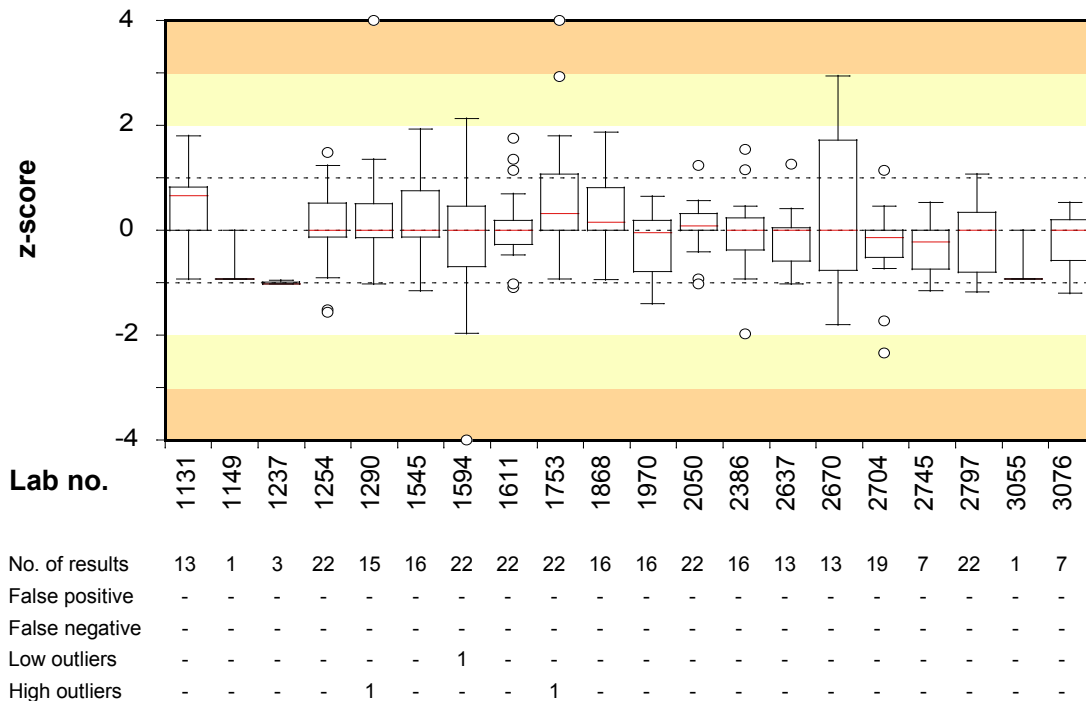
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are shown in annex B but not further evaluated. They are given explicitly to facilitate the follow-up process for the laboratories using z-scores in control charts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

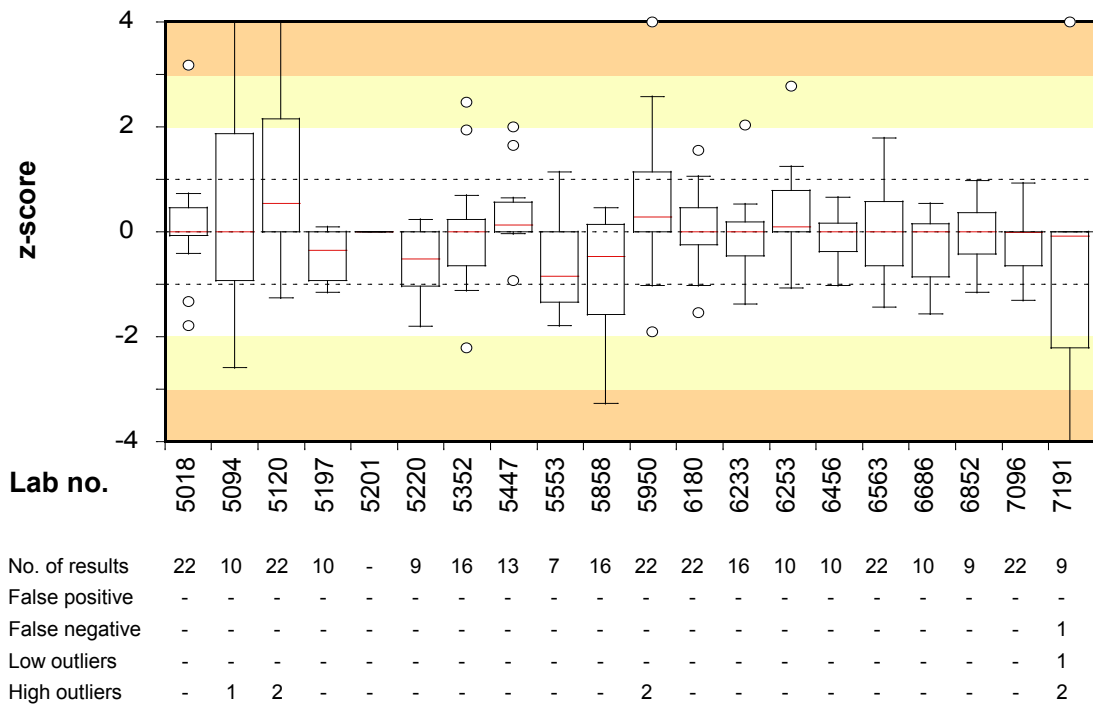
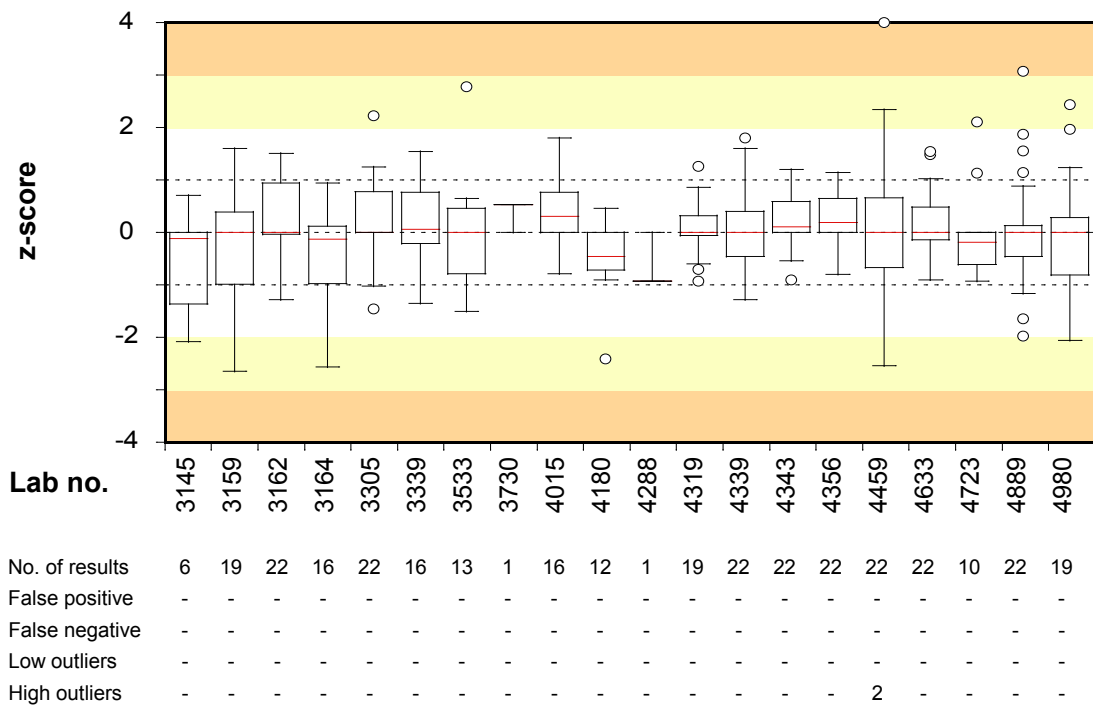
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

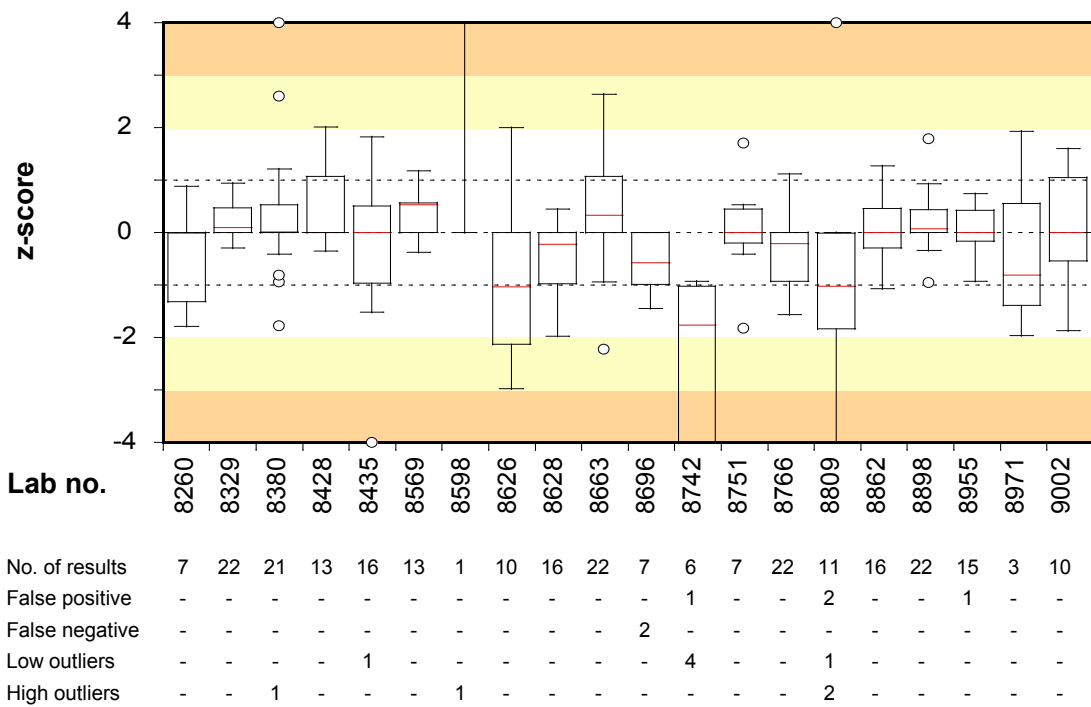
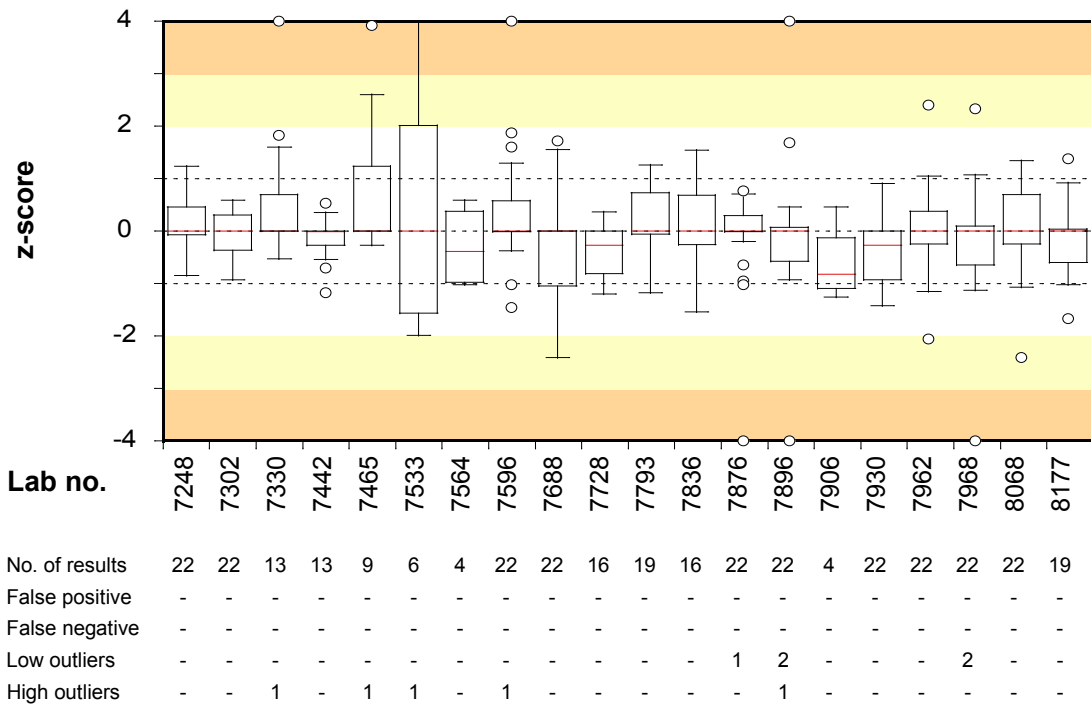
Box plots and numbers of deviating results for each participating laboratory

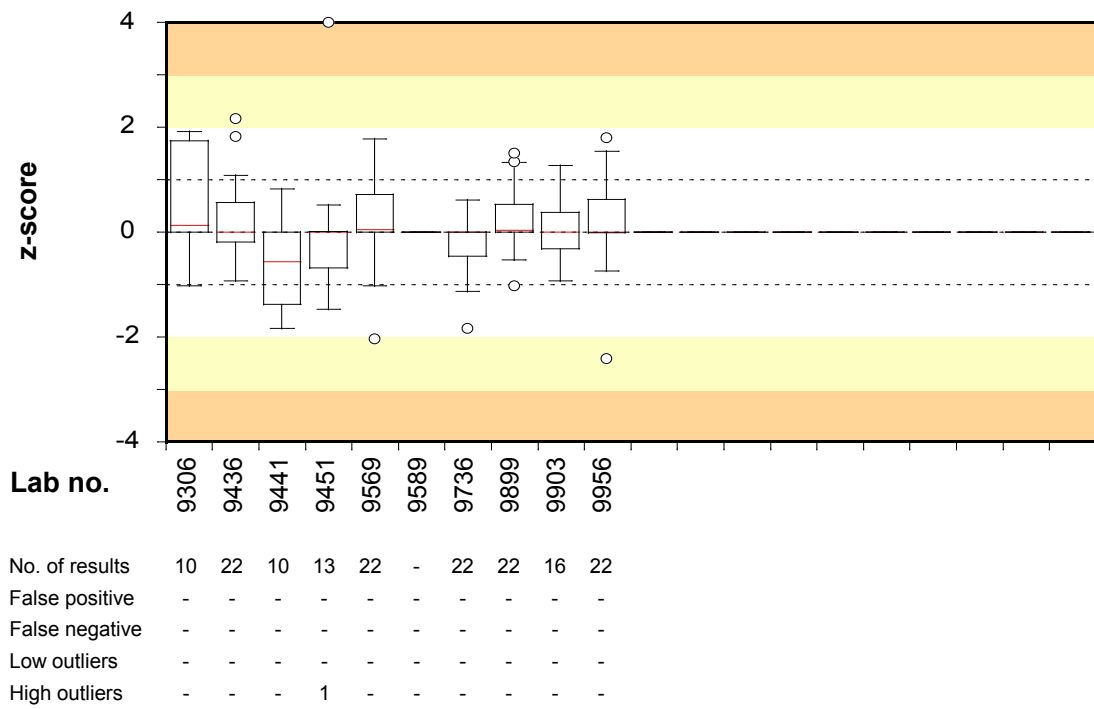
- Z-scores are calculated from the formula $z = (x - mv) / s$ (see annex A).
- Z-scores $> +4$ and < -4 have in the plots been set to $+4$ and -4 , respectively.
- False results do not generate z-scores and are not included in 'No. of results'. False positive results cannot be illustrated in the box plots.
- The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as the rest of the results.
- The numbers of false positives and false negatives are given in the table under the plots together with the numbers of outliers.
- The horizontal red 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 shown when a result is highly deviating* from the rest.
- The background is divided into coloured fields in order to simplify localization of the laboratory results.

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









Test material, quality controls and processing of data

Description of the test material

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). This round comprised three test items with different microorganism mixtures. In this round were two test items from the same original organism mixture. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition and concentrations in each mixture is 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 adapted to the EN ISO methods for analyses of drinking water referred to in the European Drinking water directive (4). Alternative methods and other standards may usually also be used without any problem.

Table 2 *Microorganisms present in the mixtures*

Mixture ¹	Microorganisms	Strain no.	cfu/100 ml ²
A	<i>Escherichia coli</i>	SLV-082	28
	<i>Escherichia coli</i>	SLV-084	
	<i>Serratia marcescens</i>	SLV-040	18
B/C	<i>Enterobacter cloacae</i>	SLV-187	160
	<i>Aeromonas hydrophila</i>	SLV-081	210
	<i>Enterococcus faecalis</i>	SLV-051	1500
	<i>Pseudomonas aeruginosa</i>	SLV-453	5
	<i>Staphylococcus warnerii</i>	SLV-189	210*

1 The links between the mixtures 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

Quality control of the test material

It is essential to have a homogeneous mixture and a uniform volume in all vials in order to allow comparison of all freeze-dried samples derived from one mixture. The volume was checked by weighing at least 12 vials of each mixture. The largest differences between vials were at most 4 mg for a mixture. The highest accepted difference is 15 mg (3%). Table 3 presents the results from the organizer in the form of concentration means (cfu) and coefficients of variation (CV) from duplicate analyses of 10 vials from each mixture. The results relate to the volume that was used for counting the colonies. According to the criteria for the CVs used they were acceptable for the mixtures to be considered homogenous. The highest accepted CV is normally 25%. For very low colony counts a higher CV is accepted. This was applied for cultural microorganisms 2d 37 °C and 3d 22 °C in mixture A.

Table 3 Contents (cfu) and measures of homogeneity (CV; coefficient of variation in per cent) in relevant sample volumes for the various parameters in the mixtures ¹

Analysis parameter <i>Method standard for analysis</i>	Mixture			
	A		B/C	
	cfu	CV	cfu	CV
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	28	4	16	8 ^a
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	22	7	b	b
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	28	4	–	–
Intestinal enterococci (MF) <i>m-Enterococcus Agar acc. to SS-EN ISO 7899-2:2000</i>	–	–	148	4 ^a
<i>Pseudomonas aeruginosa</i> (MF) <i>Pseudomonas Agar base with cetrimide and nalidixic acid according to SS-EN ISO 16288:2008</i>	–	–	5	14
Culturable microorg., 2d 37 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	< 1	75	237	2
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	c	c	226	2

1 n=10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, 13 and 14 weeks ahead of the testing round start for the mixtures A and B/C, respectively

a Result for 10 ml

b No reading was done since only some plates contained individual colonies, the rest contained none

c The analysis was not performed, only individual colonies would grow giving a very large CV

– No target organism and thus no analysis

Processing of numerical results

Most histograms have "tails" in either or both directions, due to values that do not belong to a normal distribution. Calculations are performed after square root transformations of the results that give better normal distributions by decreasing the significance of the high end "tails". Very deviating values are still 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, a few subjective adjustments

are made in order to set the right limits based on the knowledge of the mixture's contents. False results and outliers are not included in the calculations.

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 calculated from the square root transformed results and is the square root of "Mean" in Annex A, and 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 www.slv.se/absint.

References

1. Anonymous 2014. Scheme protocol, Microbiology, Drinking water & Food, 3rd ed. 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/>

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
1131	2 3 1	32	210	320	32	110	190	-	-	-	32	0	0	44	179	210	29	0	0
1149	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1254	1 2 3	-	-	-	33	130	125	17	0	0	33	0	0	26	140	140	16	0	0
1290	2 3 1	-	-	-	21	429	-	-	-	-	21	<1	<1	-	-	-	-	-	-
1545	3 2 1	20	310	380	20	80	140	20	0	0	20	0	0	-	-	-	-	-	-
1594	3 2 1	49	113	94	26	113	94	-	-	-	26	0	0	56	100	92	31	0	0
1611	2 3 1	22	165	175	22	135	140	15	118	125	22	0	0	37	126	115	22	0	0
1753	2 3 1	36	214	346	36	214	346	-	-	-	36	0	0	39	166	311	25	0	0
1868	2 1 3	26	146	154	26	146	154	-	-	-	26	0	0	30	225	182	23	0	0
1970	2 1 3	29	200	290	29	70	170	20	220	160	20	0	0	-	-	-	-	-	-
2050	2 1 3	-	-	-	25	159	154	-	-	-	25	0	0	38	156	158	27	0	0
2386	1 3 2	11	150	150	11	150	150	30	800	200	30	0	0	-	-	-	-	-	-
2637	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	34	147	161	27	<1	<1
2670	2 1 3	37	24	17	37	240	170	37	0	17	37	0	0	-	-	-	-	-	-
2704	2 3 1	-	-	-	23	100	130	-	-	-	7	0	0	34	130	130	22	<1	<1
2745	1 2 3	19	80	110	19	80	110	19	80	110	19	0	0	-	-	-	-	-	-
2797	2 1 3	29	209	300	29	155	140	15	114	103	15	0	0	31	109	118	18	0	0
3055	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	43	71	140	17	0	0
3159	1 2 3	-	-	-	18	104	200	-	-	-	13	0	0	25.4	144.5	200.5	11.1	<1	<1
3162	2 1 3	15	460	400	15	220	240	-	-	-	15	0	0	45	214	161	23	0	0
3164	1 3 2	-	-	-	26	120	163	-	-	-	23	0	0	35	88	179	23	0	0
3305	1 3 2	-	300	140	45	200	120	-	-	-	29	<1	<1	32	178	190	26	<1	<1
3339	2 3 1	-	-	-	29	140	90	-	-	-	29	0	0	-	-	-	-	-	-
3533	1 2 3	-	-	-	25	170	160	-	-	-	25	0	0	-	-	-	-	-	-
3730	2 1 3	25	210	60	-	-	-	23	0	0	-	-	-	-	-	-	-	-	-
4015	3 1 2	27	241	318	27	100	191	-	-	-	27	0	0	48	162	210	34	0	0
4180	1 2 3	-	-	-	-	-	-	-	-	-	15	0	0	-	-	-	-	-	-
4288	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	3 1 2	25	265	275	25	104	138	22	113	110	25	0	0	39	117	161	24	0	0
4339	3 2 1	15	156	260	15	156	260	12	9	150	15	0	0	33	111	122	22	0	0
4343	3 2 1	20	207	261	20	189	189	-	-	-	20	0	0	38	155	161	26	0	0
4356	2 3 1	22	150	160	22	150	160	24	38	0	22	0	0	31	160	155	26	0	0
4459	2 1 3	-	-	-	16	1700	1900	-	-	-	16	0	0	43	120	78	29	0	0
4633	3 2 1	-	-	-	33	138	152	14	0	0	33	0	0	36	135	134	25	0	0
4723	2 1 3	18	200	127	18	200	127	-	-	-	18	0	0	-	-	-	-	-	-
4889	2 3 1	-	-	-	21	50	200	-	-	-	21	0	0	35	120	100	22	0	0
4980	1 2 3	35	100	60	35	100	60	21	0	0	21	0	0	59.1	83.1	118.4	38.4	<1	<1
5018	3 1 2	12	130	180	12	130	180	-	-	-	12	0	0	37	308	135	25	0	0
5094	1 2 3	28	15800	13500	12	270	13500	6	4400	12900	6	0	0	-	-	-	-	-	-
5120	1 2 3	30	300	140	30	174	90	30	120	110	30	0	0	77	249	292	44	0	0
5197	2 3 1	-	-	-	-	-	-	-	-	-	13	0	0	-	-	-	-	-	-
5201	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5220	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	33.1	82	95.9	26.2	0	0
5352	1 2 3	44	236	162	30	136	96	18	0	0	18	0	0	-	-	-	-	-	-
5447	3 2 1	-	-	-	25	260	250	-	-	-	25	0	0	-	-	-	-	-	-
5553	2 1 3	-	-	-	12	72	108	-	-	-	12	<1,0	<1,0	-	-	-	-	-	-
5858	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	22	165	118	19	0	0
5950	1 3 2	34	90	190	49	25000	21800	25	1200	1340	9	0	0	39	140	179	29	0	0
6180	1 3 2	38	110	180	38	110	180	19	0	0	19	0	0	32	165	145	29	0	0
6233	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	27	243	156	20	<1	<1
6253	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	35	180	190	26	0	0
6456	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	38	130	145	29	0	0
6563	3 1 2	26	254	245	26	127	182	-	-	-	26	0	0	36	147	198	29	0	0
6686	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	30.6	165	165.2	16	<1	<1
6852	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	45.3	130	170	27.1	<1	<1
7096	2 1 3	-	-	-	28	130	118	-	-	-	28	0	0	34	109	109	25	<1	<1
7191	2 3 1	88	85	930	0	34	55	20	0	0	20	0	0	-	-	-	-	-	-
7248	1 2 3	35	93	135	35	93	135	24	0	0	24	0	0	45	118	168	25	0	0
7302	3 2 1	18	330	400	18	165	133	19	2	0	18	0	0	42	120	153	24	0	0
7330	1 3 2	-	-	-	-	-	-	-	-	-	26	0	0	-	-	-	-	-	-
7442	1 2 3	39	133	147	20	133	147	-	-	-	20	0	0	35	117	159	18	0	0
7465	1 2 3	35	420	320	35	420	320	20	66	61	20	0	0	-	-	-	-	-	-
7533	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	23	>23	>23	16	<1,1	<1,1
7564	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7596	2 3 1	28	230	130	28	166	130	19	0	0	19	0	0	37	157	140	30	0	0
7688	3 2 1	-	-	-	14	240	99	-	-	-	14	0	0	32	155	201	15	0	0
7728	2 1 3	-	-	-	19	123	133	-	-	-	19	0	0	-	-	-	-	-	-
7793	2 1 3	32	160	154	32	140	143	14	0	0	14	0	0	30	185	166	18	0	0
Mean					24	134	149				21	0	0	37	145	148	25	0	0
CV (%)					16	20	18				18	-	-	11	14	11	13	-	-

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		
-	-	-	-	-	-	-	-	-	-	-	-	0	177	178	-	-	-	1131	
-	-	-	-	-	-	-	-	-	-	-	-	<1	16	16	-	-	-	1149	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	180	170	1237	
-	-	-	0	1275	1650	-	-	-	0	2	8	1	213	204	1	220	217	1254	
-	-	-	<1	1218	1403	-	-	-	<1	8	9	1	-	16	<1	205	186	1290	
0	1520	1370	0	1520	1370	0	6	2	0	6	2	3	174	209	4	248	227	1545	
0	1200	1070	0	1200	1070	0	6	4	0	6	4	1	13	180	1	14	150	1594	
0	1870	1690	0	1870	1690	0	6	8	0	6	8	2	160	188	0	194	200	1611	
0	1577	1450	0	1577	1450	0	7	5	0	7	5	0	25	18	2	207	182	1753	
0	1993	1927	0	1917	1550	-	-	-	-	-	-	1	216	219	-	-	-	1868	
0	1500	1400	0	1500	1400	0	2	3	0	2	3	0	190	170	0	200	180	1970	
-	-	-	0	1227	1291	-	-	-	0	3	4	0	15	23	0	241	214	2050	
0	1150	1100	0	1150	1100	0	9	3	0	9	3	0	14	15	1	210	198	2386	
-	-	-	<1	1700	1100	-	-	-	-	-	-	<1	90	91	<1	189	183	2637	
0	106	105	0	1060	1050	-	-	-	-	-	-	7	135	132	7	159	154	2670	
-	-	-	0	1320	1060	-	-	-	-	-	-	2	180	140	1	210	150	2704	
-	-	-	-	-	-	-	-	-	-	-	-	1	77	44	-	-	-	2745	
0	1351	1365	0	1351	1365	0	2	3	0	2	3	1	206	219	2	217	202	2797	
-	-	-	-	-	-	-	-	-	-	-	-	0	60	50	-	-	-	3055	
-	-	-	-	-	-	0	4	5	0	4	5	1	198	192	0	202	165	3076	
0	1120	1046	-	-	-	0	2	1	-	-	-	-	-	-	-	-	-	3145	
-	-	-	0	1150	1500	-	-	-	-	-	-	3	168	200	3	149	144	3159	
0	15500	21300	0	1100	1700	0	4	9	0	4	9	1	175	229	1	177	200	3162	
-	-	-	-	-	-	-	-	-	-	-	-	0	10	18	0	141	140	3164	
<1	900	1500	<1	900	1500	<1	4	8	<1	4	8	<1	16	17	<1	225	215	3305	
-	-	-	0	1060	900	-	-	-	0	3	6	1	205	170	3	230	230	3339	
-	-	-	0	1000	2200	-	-	-	-	-	-	0	181	159	1	166	177	3533	
-	-	-	-	-	-	-	-	-	-	-	-	1	220	230	-	-	-	3730	
0	1680	1599	0	1375	1043	-	-	-	-	-	-	1	213	210	-	-	-	4015	
-	-	-	0	1140	1160	-	-	-	0	2	0	-	-	-	1	190	182	4180	
-	-	-	-	-	-	-	-	-	-	-	-	0	20	-	-	-	-	4288	
0	15300	14450	0	1700	1300	-	-	-	-	-	-	0	19	27	1	207	229	4319	
0	1170	1260	0	1170	1260	0	6	5	0	6	5	3	232	197	1	207	220	4339	
0	10455	9636	0	1273	1636	0	3	5	0	2	4	2	84	181	2	210	220	4343	
0	1500	1270	0	1500	1100	0	4	8	0	4	8	2	204	223	2	238	229	4356	
0	1355	1555	0	1355	1555	1	2	1	0	2	1	5	191	185	0	201	189	4459	
-	-	-	0	1282	1209	-	-	-	0	2	3	1	176	204	3	219	225	4633	
0	2182	2727	0	2000	1091	-	-	-	-	-	-	0	177	215	-	-	-	4723	
-	-	-	0	1800	1300	-	-	-	0	3	1	2	16	14	0	260	310	4889	
0	1440	1436	0	1440	1436	-	-	-	-	-	-	0	161	178	0	202	204	4980	
0	1370	1400	0	1370	1400	0	3	3	0	3	3	1	190	195	1	220	225	5018	
0	930	1260	-	-	-	0	6	2	-	-	-	0	96	184	1	260	200	5094	
0	1570	1350	0	1570	1350	0	9	5	0	9	5	0	225	244	0	279	278	5120	
-	-	-	-	-	-	-	-	-	0	2	3	0	193	186	0	193	204	5197	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5201	
-	-	-	0	1046	980.4	0	1	3.1	-	-	-	-	-	-	-	-	-	5220	
0	1390	1200	0	1390	1200	0	13	12	0	13	12	0	200	188	0	210	137	5352	
-	-	-	0	1500	1300	-	-	-	-	-	-	0	180	180	1	220	200	5447	
<1,0	825	1290	-	-	-	-	-	-	-	-	-	2	214	228	-	-	-	5553	
0	650	500	0	630	490	0	3	27	0	0	5	0	190	160	0	214	210	5858	
0	1850	1360	0	1850	1360	0	5	8	0	5	8	0	18	15	0	238	223	5950	
0	12800	11300	0	1265	1150	0	1	3	0	1	3	1	211	218	0	212	236	6180	
0	1324	1300	0	1324	1300	0	3	2	0	3	2	1	191	196	1	192	194	6233	
-	-	-	0	1000	2200	-	-	-	-	-	-	1	230	210	-	-	-	6253	
-	-	-	-	-	-	-	-	-	-	-	-	0	158	158	0	210	210	6456	
0	930	880	0	930	880	0	10	6	0	10	6	0	98	104	0	188	181	6563	
-	-	-	<1	1130	1310	-	-	-	-	-	-	<1	189	166	-	-	-	6686	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<1	175	188	6852	
-	-	-	0	1320	1260	-	-	-	0	2	2	0	184	202	0	202	207	7096	
0	2	2	-	-	-	-	-	-	-	-	-	70	73	122	115	47	177	7191	
0	1390	1243	0	1390	1243	0	3	8	0	3	8	1	186	245	1	198	212	7248	
0	1150	1382	0	1150	1382	0	2	3	0	2	3	0	173	195	1	214	215	7302	
0	1155	1113	0	1155	1113	0	4	4	0	4	4	3	265	220	25	219	263	7330	
0	1524	1338	-	-	-	-	-	-	-	-	-	1	180	143	-	-	-	7442	
0	1220	1450	0	1220	1450	-	-	-	-	-	-	-	-	-	-	-	-	7465	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	440	270	7533	
-	-	-	-	-	-	-	-	-	0	15	16	0	15	16	0	210	220	7564	
20	9600	1350	0	900	1290	0	4	50	0	4	50	3	24	20	0	260	244	7596	
0	1000	1200	0	1000	1200	-	-	-	0	4	0	1	170	180	1	160	200	7688	
-	-	-	0	1165	1263	-	-	-	0	5	2	0	187	184	0	180	165	7728	
0	1200	1400	0	1200	1400	-	-	-	-	-	-	1	200	225	2	238	243	7793	
			0	1298	1263				0	4	4	0	134	140	0	205	201	Mean	
			-	11	12				-	32	41		108	33	32	98	7	8	CV (%)

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
7836	1 3 2	-	-	-	22	200	160	-	-	-	15	0	0	-	-	-	-	-	-
7876	1 3 2	24	177	152	24	177	152	15	117	<1	24	<1	<1	43	153	119	26	<1	<1
7896	1 3 2	20	130	140	20	130	140	20	200	100	20	<1	<1	1	160	150	1	<1	<1
7906	3 1 2	32	176	124	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7930	1 3 2	15	95	104	15	95	104	-	-	-	15	0	0	29	118	106	29	0	0
7962	3 1 2	47	240	320	47	80	170	11	96	87	25	0	0	46	72	120	31	0	0
7968	1 3 2	47	103	117	28	103	117	-	-	-	28	0	0	58	116	114	23	0	0
8068	2 1 3	36	280	210	36	190	140	-	-	-	30	0	0	46	150	140	33	0	0
8177	1 2 3	32	200	320	32	120	130	21	100	88	32	0	0	25	140	150	20	0	0
8260	3 1 2	12	1867	834	12	133	200	11	<1	<1	10	<1	<1	-	-	-	-	-	-
8329	3 2 1	24	289	305	24	161	160	-	-	-	24	0	0	40	145	179	23	0	0
8380	3 1 2	36	200	320	22	200	320	-	-	-	22	<1	<1	30	200	150	20	<1	<1
8428	2 1 3	-	-	-	-	-	-	-	-	-	38	0	0	-	-	-	-	-	-
8435	3 1 2	-	-	-	19	90	80	-	-	-	19	0	0	-	-	-	-	-	-
8569	3 2 1	25	252	244	25	135	130	-	-	-	25	0	0	-	-	-	-	-	-
8598	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8626	2 3 1	31	39	124	31	23	99	6	0	0	6	0	0	-	-	-	-	-	-
8628	2 1 3	-	-	-	24	50	70	-	-	-	24	<1	<1	-	-	-	-	-	-
8663	3 1 2	47	520	420	26	150	240	25	14	31	26	0	0	30	190	160	20	0	0
8696	1 2 3	-	-	-	14	0	0	-	-	-	14	0	0	-	-	-	-	-	-
8742	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8751	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	52	79	135	27	<1	<1
8766	3 1 2	18	80	87	18	80	87	5	57	68	18	0	0	45	96	120	16	0	0
8809	2 3 1	16	4480	2780	16	4480	2780	-	-	-	8	1480	1420	-	-	-	-	-	-
8862	2 3 1	26	336	345	26	209	200	-	-	-	26	0	0	36	133	132	23	0	0
8898	2 3 1	28	240	239	28	158	138	-	-	-	28	0	0	40	151	119	25	0	0
8955	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	33	162	172	21	0	0
8971	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9002	3 2 1	20	60	80	20	220	210	-	-	-	25	0	0	-	-	-	-	-	-
9306	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	53	156	155	38	0	0
9436	2 3 1	33	264	327	33	118	173	17	11	<1	15	<1	<1	46	194	166	40	<1	<1
9441	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	44	137	107	29	<1	<1
9451	1 2 3	19	80	100	19	80	100	16	0	0	16	0	0	-	-	-	-	-	-
9569	3 2 1	29	120	190	29	120	190	<1	<1	<1	29	<1	<1	40	201	194	37	<1	<1
9589	1 2 3	20	160	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9736	2 3 1	17	242	336	17	168	158	-	-	-	17	0	0	32	149	136	24	0	0
9899	1 3 2	22	236	131	22	132	131	-	-	-	22	0	0	33	154	159	26	0	0
9903	3 2 1	22	199	378	22	154	175	16	128	160	16	0	0	-	-	-	-	-	-
9956	2 3 1	19	360	250	19	135	113	32	94	166	19	0	0	37	166	210	25	0	0

n	61	62	62	79	79	78	38	38	38	83	83	83	66	65	65	66	66	66
Min	11	24	17	0	0	0	0	0	0	6	0	0	1	71	78	1	0	0
Max	88	15800	13500	49	25000	21800	37	4400	12900	38	1480	1420	77	308	311	44	0	0
Median	26	200	195	24.5	135	143	19	12.5	8.5	21	0	0	36.5	147	150	25	0	0
Mean				24	134	149				21	0	0	37	145	148	25	0	0
CV (%)				16	20	18				18	-	-	11	14	11	13	-	-
False positive				0	0	0				0	1	1	0	0	0	0	0	0
False negative				1	1	1				0	0	0	0	0	0	0	0	0
Outliers, low				0	0	0				0	0	0	1	0	0	1	0	0
Outliers, high				0	5	4				0	0	0	1	0	2	0	0	0
Low limit OK	11	24	17	11	23	55	0	0	0	6	0	0	22	71	78	11	0	0
High limit OK	88	15800	13500	49	270	346	37	4400	12900	38	0	0	60	308	210	44	0	0

mv				4.912	11.564	12.225				4.539	0.000	0.000	6.092	12.046	12.147	4.974	0.000	0.000
($\sqrt{\text{Mean}}$)																		
s				0.808	2.280	2.172				0.809	0.000	0.000	0.654	1.734	1.305	0.622	0.000	0.000
($CV \cdot mv / 100$)																		
$u_{rel,mv}$ (%)				1.9	2.3	2.1				2.0			1.3	1.8	1.4	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	
-	-	-	0	1700	1500	-	-	-	0	1	3	0	140	210	3	210	220	7836
<1	120	1350	<1	120	1350	<1	4	2	<1	4	2	1	220	200	<1	200	220	7876
<1	1100	1800	<1	1100	1800	<1	30	2	<1	30	2	<1	33	20	1	190	210	7896
-	-	-	-	-	-	-	-	-	-	-	-	0	53	88	1	186	163	7906
0	1260	990	0	1260	990	0	3	7	0	3	7	1	192	188	1	177	188	7930
0	1250	1240	0	1250	1240	0	3	3	0	3	3	0	224	211	1	199	197	7962
0	1295	1410	0	1295	1410	0	3	4	0	3	4	0	15	15	2	13	17	7968
0	1000	1100	0	1000	1100	0	6	0	0	6	0	0	180	160	1	200	190	8068
0	1500	1000	0	1500	1000	-	-	-	-	-	-	0	190	140	0	220	200	8177
-	-	-	-	-	-	-	-	-	-	-	-	<1	191	180	-	-	-	8260
0	1441	1477	0	1441	1477	-	-	-	0	4	3	1	121	124	1	221	209	8329
-	1300	800	0	1300	800	<1	3	40	<1	3	40	1	160	130	1	210	230	8380
-	-	-	0	1245	1610	-	-	-	0	4	7	2	205	231	2	220	190	8428
-	-	-	0	1900	10	-	-	-	0	8	8	1	50	18	0	219	163	8435
0	1460	1630	0	1460	1630	-	-	-	-	-	-	1	204	225	2	230	219	8569
-	-	-	-	-	-	-	-	-	-	-	-	20	200	219	-	-	-	8598
-	-	-	-	-	-	-	-	-	-	-	-	4	121	136	0	151	158	8626
-	-	-	<1	1250	1180	-	-	-	<1	3	1	<1	16	15	<1	216	180	8628
0	6400	4000	0	2200	700	0	5	5	0	5	5	3	190	190	2	240	250	8663
-	-	-	0	1020	1100	-	-	-	-	-	-	-	182	183	-	-	-	8696
-	-	-	-	-	-	-	-	-	0.3	0.5	0.5	<1	2.3	2.2	<1	2.3	2.3	8742
-	-	-	-	-	-	-	-	-	-	-	-	1	149	189	-	-	-	8751
0	1387	1745	0	1234	1273	0	4	2	0	4	2	0	191	238	0	200	238	8766
0	640	850	0	640	850	-	-	-	-	-	-	0	150	175	0	90	200	8809
0	6200	2800	0	1000	1500	-	-	-	-	-	-	0	119	119	-	-	-	8862
0	1200	1318	0	1200	1318	0	10	5	0	10	5	1	194	198	1	208	193	8898
10	1270	1273	10	1270	1273	-	-	-	0	6	3	0	210	193	1	220	205	8955
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	155	176	8971
0	800	1000	0	800	1000	-	-	-	-	-	-	3	170	110	-	-	-	9002
-	-	-	-	-	-	-	-	-	-	-	-	0	247	187	0	206	260	9306
<1	1900	1100	<1	1900	1100	<1	4	5	<1	4	5	<1	167	218	1	188	195	9436
-	-	-	-	-	-	-	-	-	-	-	-	<1	50	61	<1	158	156	9441
0	1260	870	0	1300	870	-	-	-	-	-	-	20	180	200	1	220	180	9451
<1	1900	810	<1	1300	740	<1	4	10	<1	4	10	1	195	180	<1	218	204	9569
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9589
0	1345	1162	0	1290	1135	1	4	3	0	4	3	1	160	134	1	158	167	9736
0	13091	11864	0	1727	1682	0	7	7	0	7	7	1	174	197	0	249	203	9899
0	1159	1273	0	1159	1273	0	8	5	0	8	5	0	200	218	2	202	190	9903
0	1490	1228	0	1490	1228	0	9	0	0	9	0	1	225	237	2	238	241	9956

	62	63	63	79	80	80	45	45	45	60	60	60	99	99	99	85	86	86	n
	0	2	2	0	120	10	0	1	0	0	0	0	0	2.3	2.2	0	2.3	2.3	Min
	20	15500	21300	10	2200	2200	1	30	50	0.3	30	50	70	265	245	115	440	310	Max
	0	1351	1338	0	1273	1273	0	4	5	0	4	4	1	178.5	182	1	209	200	Median
				0	1298	1263				0	4	4	0	134	140	0	205	201	Mean
				-	11	12				-	32	41	108	33	32	98	7	8	CV (%)
				1	0	0				1	0	0	0	0	0	0	0	0	False pos.
				0	0	0				0	0	0	0	0	0	0	0	0	False neg.
				0	1	1				0	0	0	0	1	1	0	5	2	Outliers <
				0	0	0				0	1	2	3	0	0	2	1	0	Outliers >
	0	2	2	0	630	490	0	1	0	0	0	0	0	10	10	0	141	137	Low limit
	20	15500	21300	0	2200	2200	1	30	50	0	13	12	7	265	265	7	279	310	High limit

	0.000	36.027	35.539				0.000	2.000	1.923	0.634	11.587	11.820	0.688	14.332	14.178				mv
	0.000	4.128	4.097				0.000	0.649	0.796	0.683	3.768	3.785	0.678	0.961	1.117				s
		1.3	1.3					4.2	5.4	11.0	3.3	3.2	10.8	0.7	0.9				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							0.922	-0.472	0.718				1.381	0.000	0.000	0.827	0.769	1.796	0.661	0.000	0.000
1149																					
1237																					
1254							1.030	-0.071	-0.481				1.489	0.000	0.000	-1.518	-0.123	-0.242	-1.565	0.000	0.000
1290							-0.407	4.000				0.053	0.000	0.000							
1545							-0.544	-1.149	-0.181			-0.083	0.000	0.000							
1594							0.232	-0.410	-1.165			0.691	0.000	0.000	2.126	-1.180	-1.958	0.955	0.000	0.000	
1611							-0.274	0.024	-0.181			0.187	0.000	0.000	-0.015	-0.473	-1.091	-0.455	0.000	0.000	
1753							1.346	1.344	2.936			1.805	0.000	0.000	0.233	0.484	4.000	0.042	0.000	0.000	
1868							0.232	0.228	0.085			0.691	0.000	0.000	-0.940	1.704	1.029	-0.286	0.000	0.000	
1970							0.586	-1.403	0.375			-0.083	0.000	0.000							
2050							0.109	0.458	0.085			0.569	0.000	0.000	0.110	0.256	0.324	0.357	0.000	0.000	
2386							-1.973	0.300	0.011			1.159	0.000	0.000							
2637															-0.400	0.046	0.415	0.357	0.000	0.000	
2670							1.449	1.723	0.375			1.907	0.000	0.000							
2704							-0.143	-0.686	-0.379			-2.339	0.000	0.000	-0.400	-0.371	-0.571	-0.455	0.000	0.000	
2745							-0.684	-1.149	-0.800			-0.223	0.000	0.000							
2797							0.586	0.388	-0.181			-0.823	0.000	0.000	-0.802	-0.926	-0.984	-1.175	0.000	0.000	
3055																					
3076																					
3145																					
3159							-0.828	-0.599	0.883			-1.154	0.000	0.000	0.711	-2.087	-0.242	-1.367	0.000	0.000	
3162							-1.285	1.434	1.505			-0.823	0.000	0.000	-1.609	-0.014	1.542	-2.639	0.000	0.000	
3164							0.232	-0.268	0.250			0.941	1.490	0.415	0.941	1.490	0.415	-0.286	0.000	0.000	
3305							2.222	1.131	-0.585			0.317	0.000	0.000	-0.270	-1.537	0.944	-0.286	0.000	0.000	
3339							0.586	0.117	-1.261			1.045	0.000	0.000	-0.666	0.748	1.254	0.201	0.000	0.000	
3533							0.109	0.647	0.195			0.569	0.000	0.000							
3730																					
4015							0.352	-0.686	0.735			0.811	0.000	0.000	1.277	0.394	1.796	1.378	0.000	0.000	
4180												-0.823	0.000	0.000							
4288																					
4319							0.109	-0.599	-0.220			0.569	0.000	0.000	0.233	-0.709	0.415	-0.120	0.000	0.000	
4339							-1.285	0.406	1.796			-0.823	0.000	0.000	-0.532	-0.871	-0.844	-0.455	0.000	0.000	
4343							-0.544	0.958	0.701			-0.083	0.000	0.000	0.110	0.233	0.415	0.201	0.000	0.000	
4356							-0.274	0.300	0.195			0.187	0.000	0.000	-0.802	0.348	0.232	0.201	0.000	0.000	
4459							-1.128	4.000	4.000			-0.666	0.000	0.000	0.711	-0.629	-2.540	0.661	0.000	0.000	
4633							1.030	0.080	0.048			1.489	0.000	0.000	-0.141	-0.246	-0.438	0.042	0.000	0.000	
4723							-0.828	1.131	-0.440			-0.367	0.000	0.000							
4889							-0.407	-1.971	0.883			0.053	0.000	0.000	-0.270	-0.629	-1.645	-0.455	0.000	0.000	
4980							1.243	-0.686	-2.062			0.053	0.000	0.000	2.438	-1.689	-0.970	1.965	0.000	0.000	
5018							-1.791	-0.071	0.549			-1.328	0.000	0.000	-0.015	3.174	-0.405	0.042	0.000	0.000	
5094							-1.791	2.135	4.000			-2.582	0.000	0.000							
5120							0.700	0.713	-1.261			1.159	0.000	0.000	4.000	2.153	3.785	2.667	0.000	0.000	
5197												-1.154	0.000	0.000							
5201																					
5220																					
5352							0.700	0.043	-1.118			-0.367	0.000	0.000	-0.518	-1.724	-1.804	0.233	0.000	0.000	
5447							0.109	2.000	1.652			0.569	0.000	0.000							
5553							-1.791	-1.351	-0.844			-1.328	0.000	0.000							
5858															-2.143	0.461	-0.984	-0.988	0.000	0.000	
5950							2.583	4.000	4.000			-1.902	0.000	0.000	0.233	-0.123	0.944	0.661	0.000	0.000	
6180							1.550	-0.472	0.549			-0.223	0.000	0.000	-0.666	0.461	-0.081	0.661	0.000	0.000	
6233															-1.370	2.043	0.262	-0.806	0.000	0.000	
6253															-0.270	0.791	1.254	0.201	0.000	0.000	
6456															0.110	-0.371	-0.081	0.661	0.000	0.000	
6563							0.232	-0.129	0.583			0.691	0.000	0.000	-0.141	0.046	1.474	0.661	0.000	0.000	
6686															-0.857	0.461	0.541	-1.565	0.000	0.000	
6852															0.975	-0.371	0.683	0.373	0.000	0.000	
7096							0.470	-0.071	-0.627			0.929	0.000	0.000	-0.400	-0.926	-1.308	0.042	0.000	0.000	
7191								-2.515	-2.214			-0.083	0.000	0.000							
7248							1.243	-0.843	-0.279			0.444	0.000	0.000	0.941	-0.682	0.624	0.042	0.000	0.000	
7302							-0.828	0.562	-0.319			-0.367	0.000	0.000	0.594	-0.629	0.170	-0.120	0.000	0.000	
7330												0.691	0.000	0.000							
7442							-0.544	-0.014	-0.046			-0.083	0.000	0.000	-0.270	-0.709	0.354	-1.175	0.000	0.000	
7465							1.243	3.917	2.608			-0.083	0.000	0.000							
7533															-1.982			-1.565	0.000	0.000	
7564																					
7596							0.470	0.579	-0.379			-0.223	0.000	0.000	-0.015	0.279	-0.242	0.809	0.000	0.000	
7688							-1.447	1.723	-1.048			-0.985	0.000	0.000	-0.666	0.233	1.555	-1.769	0.000	0.000	
7728							-0.684	-0.208	-0.319			-0.223	0.000	0.000							
7793							0.922	0.117	-0.123			-0.985	0.000	0.000	-0.940	0.897	0.564	-1.175	0.000	0.000	
7836							-0.274	1.131	0.195			-0.823	0.000	0.000							
7876							-0.016	0.763	0.048			0.444	0.000	0.000	0.711	0.187	-0.949	0.201	0.000	0.000	
7896							-0.544	-0.071	-0.181			-0.083	0.000	0.000	-4.000	0.348	0.077	-4.000	0.000	0.000	
7906																					
7930							-1.285	-0.797	-0.933			-0.823	0.000	0.000	-1.081	-0.682	-1.419	0.661	0.000	0.000	
7962							2.405	-1.149	0.375			0.569	0.000	0.000	1.054	-2.053	-0.914	0.955	0.000	0.000	
7968							0.470	-0.621	-0.648			0.929	0.000	0.000	2.328	-0.735	-1.126	-0.286	0.000	0.000	
8068							1.346	0.974	-0.181			1.159	0.000	0.000	1.054	0.116	-0.242	1.239	0.000	0.000	
8177							0.922	-0.268	-0.379			1.381	0.000	0.000	-1.670	-0.123	0.077	-0.806	0.000	0.000	
8260							-1.791	-0.014	0.883			-1.701	0.000	0.000							
8329							-0.016	0.493	0.195			0.444	0.000								

positive results can no z-scores be calculated. Z-scores from outliers are not real z-scores but a practical means to express also the results from the outliers. Very low and high values are here limited to -4 and +4, respectively.

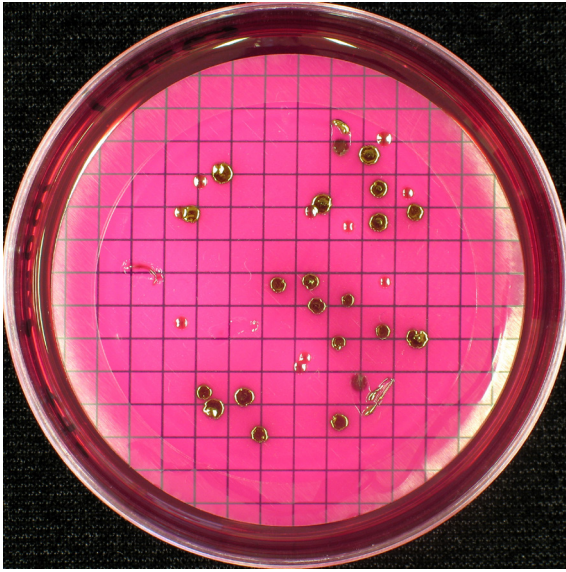
Susp. intestinal enterococci (MF)			Intestinal enterococci (MF)			Susp. <i>Pseudomonas aeruginosa</i> (MF)			<i>Pseudomonas aeruginosa</i> (MF)			Total plate count 22 °C, 3 days			Total plate count 36±2 °C, 2 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
																		1131
																		1149
			0.000	-0.077	1.240				0.000	-0.903	1.138	0.535						1237
			0.000	-0.273	0.468				0.000	1.276	1.354	0.535						1254
			0.000	0.717	0.360				0.000	0.692	-0.639	1.606						1290
			0.000	-0.336	-0.690				0.000	0.692	0.097	0.535						1545
			0.000	1.748	1.360				0.000	0.692	1.138	1.141						1594
			0.000	0.893	0.620				0.000	0.994	0.394	-0.928						1611
			0.000	1.879	0.935				0.000			0.535						1753
			0.000	0.655	0.458				0.000	-0.903	-0.239	-0.928						1868
			0.000	-0.242	0.096				0.000	-0.413	0.097	-0.928						1970
			0.000	-0.512	-0.579				0.000	1.540	-0.239	-0.928						2050
			0.000	1.261	-0.579				0.000			-0.928						2386
			0.000	-0.840	-0.765				0.000			2.943						2637
			0.000	0.074	-0.728				0.000			1.141						2670
									0.000			0.535						2704
									0.000			-0.928						2745
			0.000	0.177	0.343				0.000	-0.903	-0.239	0.535						2797
									0.000	0.000	0.394	0.535						3055
												-0.928						3076
												0.535						3145
			0.000	-0.512	0.779				0.000			1.606						3159
			0.000	-0.693	1.389				0.000	0.000	1.354	0.535						3162
									0.000			-0.928						3164
			0.000	-1.460	0.779				0.000	0.000	1.138	-0.928						3305
			0.000	-0.840	-1.352				0.000	-0.413	0.662	0.535						3339
			0.000	-1.067	2.774				0.000			-0.928						3533
									0.000			0.535						3730
			0.000	0.255	-0.792				0.000			0.535						4015
			0.000	-0.548	-0.361				0.000	-0.903	-2.415	-0.928						4180
												-0.928						4288
			0.000	1.261	0.126				0.000	0.692	0.394	1.606						4319
			0.000	-0.441	-0.010				0.000	-0.903	0.097	1.141						4339
			0.000	-0.084	1.198				0.000	0.000	1.138	1.141						4343
			0.000	0.655	-0.579				0.000	-0.903	-1.159	2.344						4459
			0.000	0.190	0.951				0.000	-0.903	-0.239	0.535						4633
			0.000	-0.054	-0.188				0.000			-0.928						4723
			0.000	2.106	-0.612				0.000	-0.413	-1.159	1.141						4889
			0.000	1.550	0.126				0.000			-0.928						4980
			0.000	0.465	0.575				0.000	-0.413	-0.239	0.535						5018
			0.000	0.239	0.458				0.000			-0.928						5094
									0.000	1.540	0.394	-0.928						5120
									0.000	-0.903	-0.239	-0.928						5197
												-0.928						5201
			0.000	-0.893	-1.032				0.000			-0.928						5220
			0.000	0.304	-0.219				0.000	2.473	1.937	-0.928						5352
			0.000	0.655	0.126							-0.928						5447
												1.141						5553
			0.000	-2.647	-3.271				0.000	-3.081	0.394	-0.928						5858
			0.000	1.692	0.327				0.000	0.363	1.138	-0.928						5950
			0.000	-0.111	-0.397				0.000	-1.541	-0.239	0.535						6180
			0.000	0.087	0.126				0.000	-0.413	-0.639	0.535						6233
			0.000	-1.067	2.774				0.000			0.535						6253
									0.000			-0.928						6456
			0.000	-1.340	-1.434				0.000	1.790	0.662	-0.928						6563
			0.000	-0.584	0.160							-0.928						6686
												-0.928						6852
			0.000	0.074	-0.010				0.000	-0.903	-0.639	-0.928						7096
												4.000						7191
			0.000	0.304	-0.069				0.000	-0.413	1.138	0.535						7248
			0.000	-0.512	0.399				0.000	-0.903	-0.239	-0.928						7302
			0.000	-0.495	-0.532				0.000	0.000	0.097	1.606						7330
												0.535						7442
			0.000	-0.266	0.620							-0.928						7465
												-0.928						7533
												1.606						7564
			0.000	-1.460	0.092				0.000	0.000	4.000	1.606						7596
			0.000	-1.067	-0.219				0.000	0.000	-2.415	0.535						7688
			0.000	-0.459	0.000				0.000	0.363	-0.639	-0.928						7728
			0.000	-0.336	0.458				0.000			0.535						7793
			0.000	1.261	0.779				0.000	-1.541	-0.239	-0.928						7836
			0.000	-4.000	0.294				0.000	0.000	-0.639	0.535						7876
			0.000	-0.693	1.681				0.000	4.000	-0.639	-0.928						7896
												-0.928						7906
			0.000	-0.129	-0.995				0.000	-0.413	0.909	0.535						7930
			0.000	-0.163	-0.080				0.000	-0.413	-0.239	-0.928						7962
			0.000	-0.010	0.491				0.000	-0.413	0.097	-0.928						7968
			0.000	-1.067	-0.579				0.000	0.692	-2.415	-0.928						8068
			0.000	0.655	-0.956							-0.928						8177
												-0.928						8260
			0.000	0.468	0.706				0.000	0.000	-0.239	0.535						8329
			0.007	-1.771					0.000	-0.413	4.000	0.535						8380
			0.000	-0.180	1.119				0.000	0.000	0.909	1.141						8428

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
8435					-0.684	-0.911	-1.511				-0.223	0.000	0.000						
8569					0.109	0.024	-0.379				0.569	0.000	0.000						
8598																			
8626					0.812	-2.969	-1.048				-2.582	0.000	0.000						
8628					-0.016	-1.971	-1.777				0.444	0.000	0.000						
8663					0.232	0.300	1.505				0.691	0.000	0.000	-0.940	1.003	0.384	-0.806	0.000	0.000
8696					-1.447						-0.985	0.000	0.000						
8742																			
8751																			
8766					-0.828	-1.149	-1.334				-0.367	0.000	0.000	1.710	-1.821	-0.405	0.357	0.000	0.000
8809					-1.128	4.000	4.000				-2.114			0.941	-1.296	-0.914	-1.565	0.000	0.000
8862					0.232	1.269	0.883				0.691	0.000	0.000	-0.141	-0.296	-0.504	-0.286	0.000	0.000
8898					0.470	0.441	-0.220				0.929	0.000	0.000	0.355	0.140	-0.949	0.042	0.000	0.000
8955														-0.532	0.394	0.741	-0.629	0.000	0.000
8971																			
9002					-0.544	1.434	1.044				0.569	0.000	0.000						
9306														1.815	0.256	0.232	1.913	0.000	0.000
9436					1.030	-0.308	0.427				-0.823	0.000	0.000	1.054	1.086	0.564	2.171	0.000	0.000
9441														0.827	-0.197	-1.382	0.661	0.000	0.000
9451					-0.684	-1.149	-1.024				-0.666	0.000	0.000						
9569					0.586	-0.268	0.718				1.045	0.000	0.000	0.355	1.229	1.364	1.782	0.000	0.000
9589																			
9736					-0.975	0.613	0.159				-0.514	0.000	0.000	-0.666	0.093	-0.372	-0.120	0.000	0.000
9899					-0.274	-0.033	-0.359				0.187	0.000	0.000	-0.532	0.210	0.354	0.201	0.000	0.000
9903					-0.274	0.371	0.462				-0.666	0.000	0.000						
9956					-0.684	0.024	-0.734				-0.223	0.000	0.000	-0.015	0.484	1.796	0.042	0.000	0.000
n					78	78	77	0	0	0	83	82	82	66	65	65	66	66	66
Min					-1.973	-2.969	-2.214				-2.582	0.000	0.000	-4.000	-2.087	-2.540	-4.000	0.000	0.000
Max					2.583	4.000	4.000				2.008	0.000	0.000	4.000	3.174	4.000	2.667	0.000	0.000
Median					0.047	0.033	0.011				0.053	0.000	0.000	-0.078	0.046	0.170	0.042	0.000	0.000
Mean					0.000	0.255	0.208				0.000	0.000	0.000	0.000	0.000	0.120	-0.061	0.000	0.000
SD					1.000	1.378	1.321				1.000	0.000	0.000	1.209	1.000	1.195	1.108	0.000	0.000
z<-3					0	0	0				0	0	0	1	0	0	1	0	0
-3≤z<-2					0	2	2				4	0	0	1	2	1	1	0	0
-2<z≤3					3	2	3				1	0	0	3	2	0	2	0	0
z>3					0	5	4				0	0	0	1	1	2	0	0	0

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			
			0.000	1.832	-4.000				0.000	1.276	1.138	0.535				-1.016	0.486	-1.263	8435	
			0.000	0.529	1.180							0.535				1.071	0.868	0.556	8569	
												4.000							8598	
												1.998				-1.016	-2.128	-1.440	8626	
			0.000	-0.163	-0.290				0.000	-0.413	-1.159	-0.928				-1.016	0.380	-0.682	8628	
			0.000	2.635	-2.217				0.000	0.363	0.394	1.606				1.071	1.208	1.463	8663	
			0.000	-0.991	-0.579														8696	
												-1.992	-1.527	-0.928		-1.016	-4.000	-4.000	8742	
												0.535							8751	
			0.000	-0.218	0.034				0.000	0.000	-0.639	-0.928				-1.016	-0.198	1.119	8766	
			0.000	-2.599	-1.558							-0.928				-1.016	-4.000	-0.032	8809	
			0.000	-1.067	0.779							-0.928							8862	
			0.000	-0.336	0.187				0.000	1.790	0.394	0.535				0.460	0.094	-0.256	8898	
				-0.094	0.034				0.000	0.692	-0.239	-0.928				0.460	0.521	0.125	8955	
																1.935	-1.959	-0.816	8971	
			0.000	-1.876	-0.956							1.606							9002	
												-0.928				-1.016	0.022	1.743	9306	
			0.000	1.832	-0.579				0.000	0.000	0.394	-0.928				0.460	-0.646	-0.192	9436	
												-0.928				-1.016	-1.834	-1.512	9441	
			0.000	0.007	-1.475							4.000				0.460	0.521	-0.682	9451	
			0.000	0.007	-2.035				0.000	0.000	1.557	0.535				-1.016	0.451	0.094	9569	
																			9589	
			0.000	-0.027	-0.451				0.000	0.000	-0.239	0.535				0.460	-1.834	-1.124	9736	
			0.000	1.340	1.336				0.000	0.994	0.909	0.535				-1.016	1.507	0.062	9899	
			0.000	-0.480	0.034				0.000	1.276	0.394	-0.928				1.071	-0.124	-0.353	9903	
			0.000	0.623	-0.121				0.000	1.540	-2.415	0.535				1.071	1.140	1.205	9956	
0	0	0	78	80	80	0	0	0	59	60	60	99	0	0	85	86	86		n	
			0.000	-4.000	-4.000				0.000	-3.081	-2.415	-0.928				-1.016	-4.000	-4.000		Min
			0.000	2.635	2.774				0.000	4.000	4.000	4.000				4.000	4.000	3.070		Max
			0.000	-0.089	0.034				0.000	0.000	0.097	0.535				0.460	0.058	-0.032		Median
			0.000	-0.050	-0.050				0.000	0.067	0.133	0.121				0.094	-0.186	-0.093		Mean
			0.000	1.090	1.090				0.000	1.118	1.221	1.202				1.161	1.423	1.159		SD
			0	1	2				0	1	0	0				0	5	2		Summa
			0	2	2				0	0	4	0				0	3	2		13
			0	2	2				0	1	0	2				1	1	2		26
			0	0	0				0	1	2	3				2	1	1		27
																				23

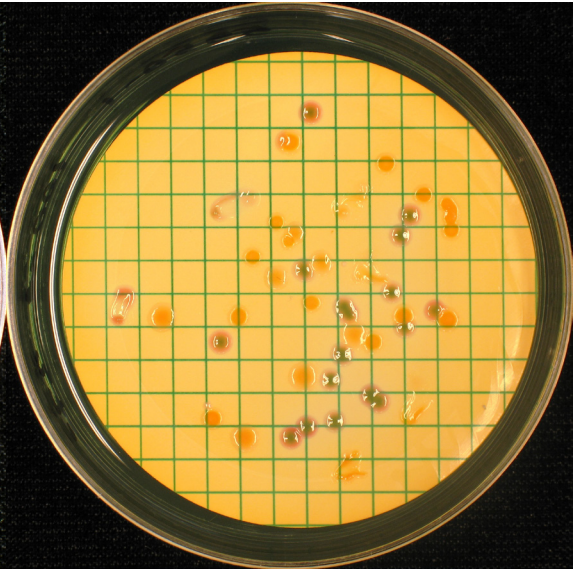
Mixture A

m-Endo Agar LES, 37 °C



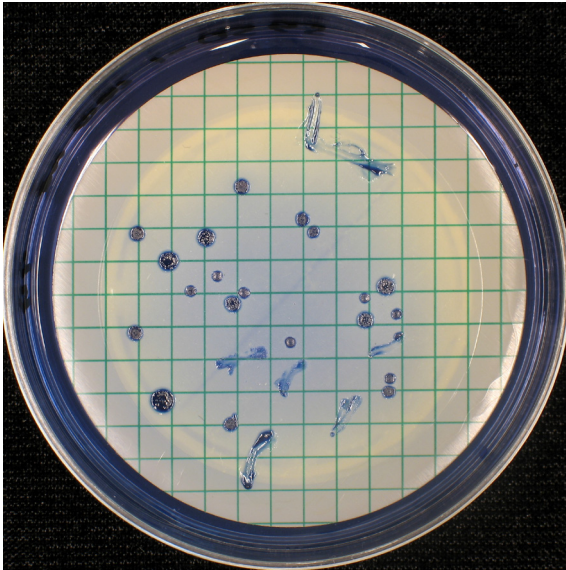
100 ml

m-Lactose TTC Agar, 37 °C



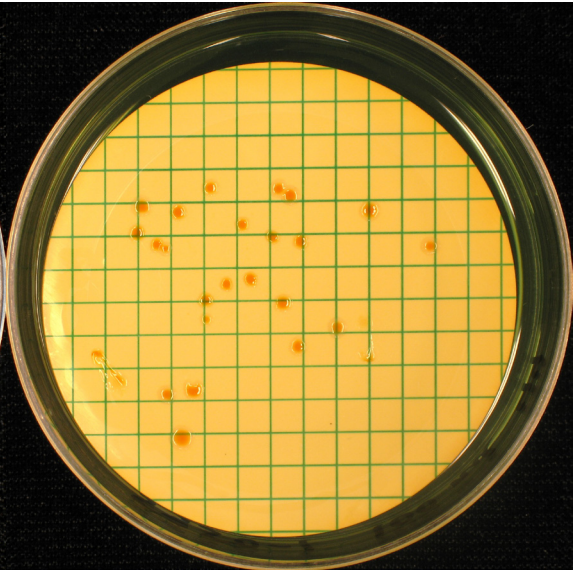
100 ml

m-FC Agar, 44 °C



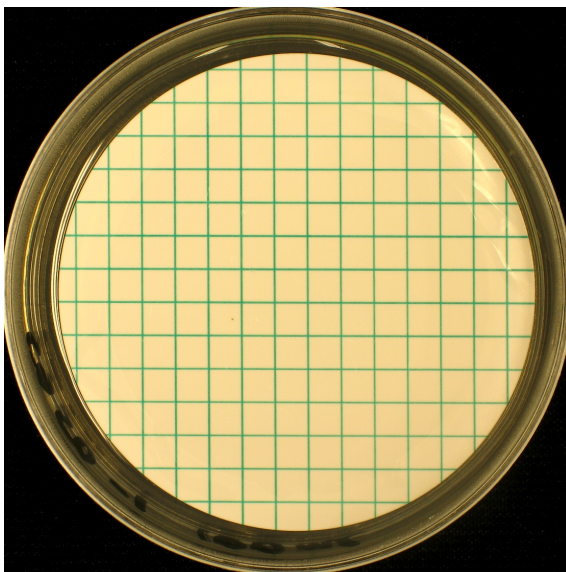
100 ml

m-Lactose TTC Agar, 44 °C



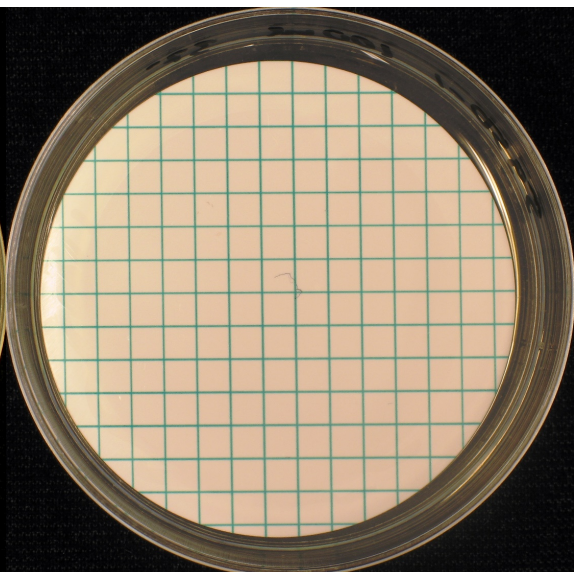
100 ml

m-Enterococcus Agar, 37 °C



100 ml, 2 days

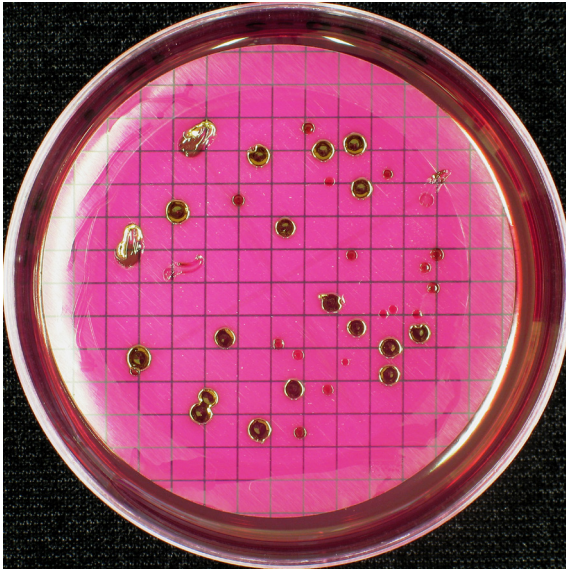
m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

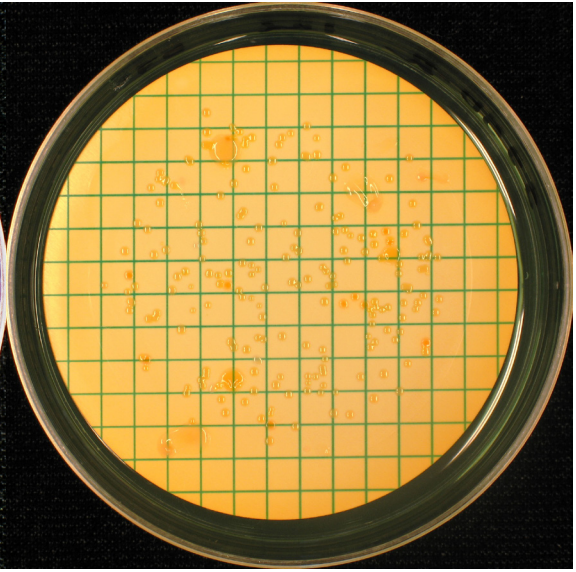
Mixture B = Mixture C

m-Endo Agar LES, 37 °C



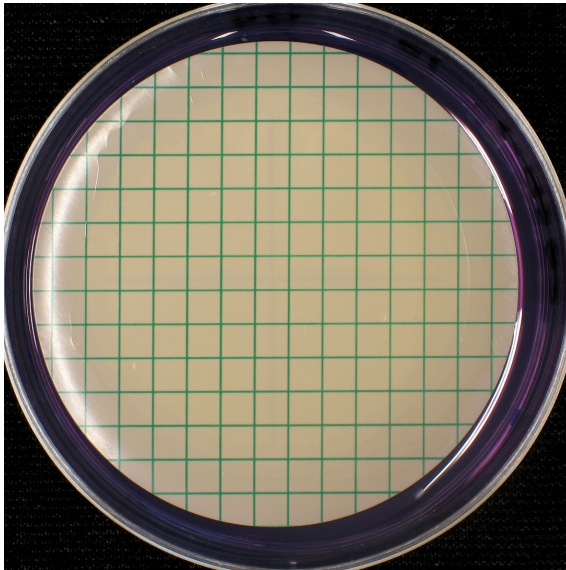
10 ml

m-Lactose TTC Agar, 37 °C



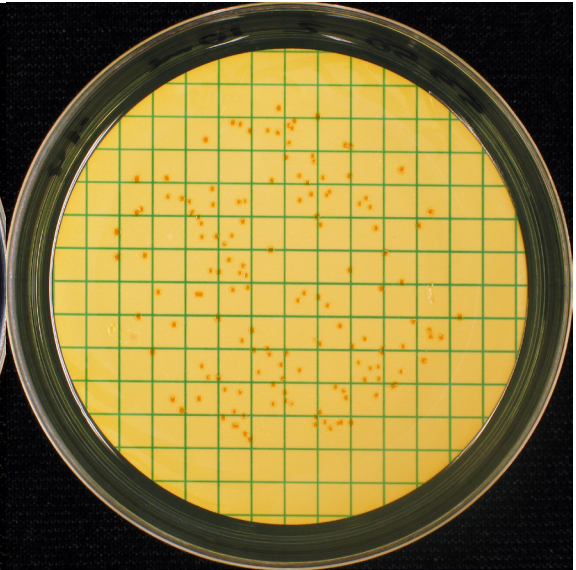
1 ml

m-FC Agar, 44 °C



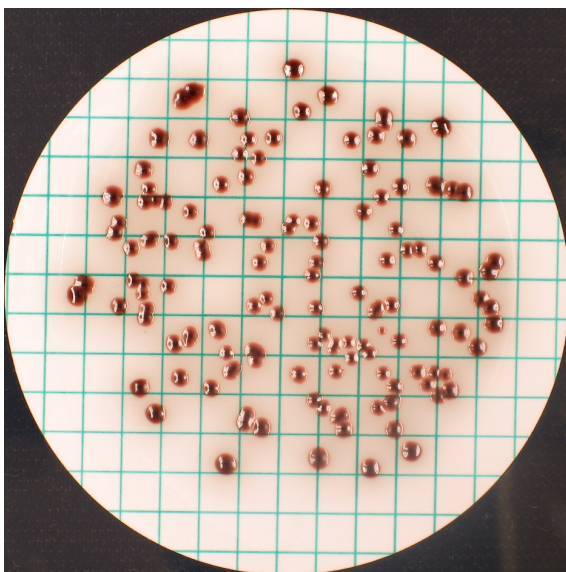
10 ml

m-Lactose TTC Agar, 44 °C



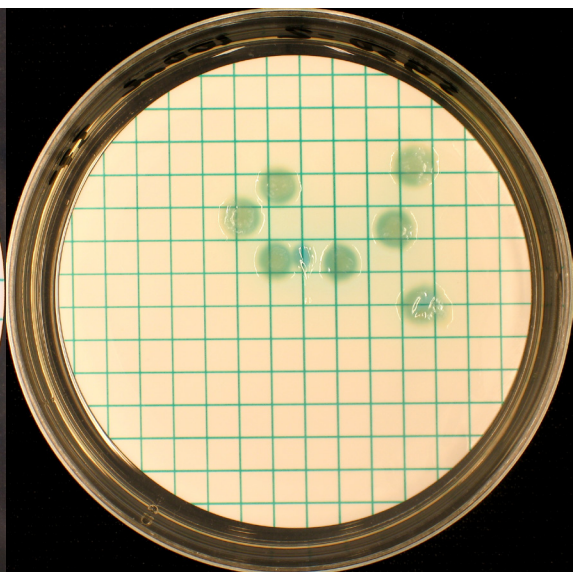
10 ml

m-Enterococcus Agar, 37 °C



10 ml, 2 days on BEAA

m-Pseudomonas CN Agar, 37 °C



100 ml, 2 days

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Boriak

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Irina Boriak

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: www.slv.se/absint



1457
ISO/IEC 17043

The National Food Agency's reference material

As a complement to the proficiency testing, 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: www.slv.se/RM