

Drinking Water Microbiology

March 2017

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Drinking water Microbiology
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Parameters included

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

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

Clostridium perfringens with MF

Actinomycetes with MF

Moulds with MF

Yeasts with MF

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

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Abbreviations and explanations

Microbiological media

ACTA	Actinomycete Isolation Agar (according to SS 028212)
CCA	Chromocult Coliform Agar [®] (Merck; according to EN ISO 9308-1:2014)
Colilert	Colilert [®] Quanti-Tray [®] (IDEXX Inc.; according to EN ISO 9308-2:2014)
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 (according to SS 028167)
PAB/TSC/SFP	Tryptose Sulfite Cycloserine Agar (acc. to ISO/CD 6461-2:2002)
RBCC	Rose Bengal Agar with both chlortetracycline and chloramphenicol (according to SS 028192)
YeA	Yeast extract Agar (according to EN ISO 6222:1999)

Other abbreviations

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

Legend to method comparison tables

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

Explanations to histograms with accepted and deviating results

	result without remark
	false negative result
	outlier
↓ 34	average without deviating results

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General information on results evaluation

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 methods 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 inconsistency 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. Thus, to get an as appropriate evaluation as possible of the results, it is important that correct standards and method details are reported.

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in tables together with the group means etc. The mean and measure of dispersion is not shown for groups with 4 or fewer results, more than exceptionally when it is specifically mentioned. However, all results are shown in the method histogram when possible.

The histograms and calculation of outliers are described on page 30 under "Processing of numerical results" with further reference to the scheme protocol (1).

Results of the PT round

General outcome

Test items were sent to 86 laboratories, 34 in Sweden, 48 in other Nordic countries (Faeroe Islands, Greenland and Åland included), 3 more from EU, 0 from the rest of Europe and 1 from countries outside Europe. Results were reported from 84 laboratories.

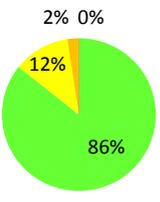
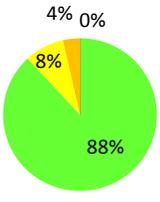
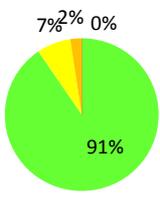
The percentages of false results and outliers are compiled in **table 1**. These deviating results are excluded in most calculations.

Microorganisms and parameters of analyses are also compiled in table 1. For the MF analyses the parameters *suspected* coliform bacteria and thermotolerant coliform bacteria 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** and results for each laboratory are also shown on our website after logging in (www2.slv.se/absint).

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

Table 1 Microorganisms in each 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	507			518			516		
No. of deviating results *	14 (3%)			13 (3%)			10 (2%)		
Microorganisms	<i>Enterobacter aerogenes</i> <i>Cronobacter sakazakii</i> <i>Hanseniaspora uvarum</i> <i>Phialophora malorum</i> <i>Pseudomonas fluorescens</i>			<i>Citrobacter freundii</i> <i>Escherichia coli</i> <i>Clostridium bifermentans</i> <i>Streptomyces sp.</i> <i>Staphylococcus saprophyticus</i>			<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Clostridium perfringens</i> <i>Cladosporium cladosporoides</i> <i>Staphylococcus saprophyticus</i>		
Analysis	Målorganism	F%	X%	Målorganism	F%	X%	Målorganism	F%	X%
Coliform bacteria (MF)	<i>E. aerogenes</i> <i>C. sakazakii</i>	1	0	<i>C freundii</i> <i>E. coli</i>	1	3	<i>E. coli</i> <i>K. pneumoniae</i>	0	1
Susp. thermotolerant coliform bact. (MF)	[<i>E. aerogenes</i>] [<i>C. sakazakii</i>]	–	–	<i>E. coli</i>	–	–	<i>E. coli</i> <i>K. pneumoniae</i>	–	–
<i>E. coli</i> (MF)	[<i>C. sakazakii</i>]	1	–	<i>E. coli</i>	7	1	<i>E. coli</i> [<i>K. pneumon.</i>]	3	0
Coliform bacteria (rapid method)	<i>E. aerogenes</i> <i>C. sakazakii</i>	0	4	<i>C freundii</i> <i>E. coli</i>	0	0	<i>E. coli</i> <i>K. pneumoniae</i>	0	5
<i>E. coli</i> (rapid meth.)	–	0	0	<i>E. coli</i>	0	0	<i>E. coli</i>	2	0
Presumptive <i>C. perfringens</i> (MF)	–	2	–	<i>C. bifermentans</i>	0	0	<i>C. perfringens</i>	0	0
<i>Clostridium perfringens</i> (MF)	–	0	–	[<i>C. biferment.</i>]	3	0	<i>C. perfringens</i>	3	0
Actinomycetes (MF) 25 °C	–	0	–	<i>Streptomyces sp.</i>	0	0	–	3	–
Moulds (MF) 25 °C	<i>Ph. malorum</i>	14	3	–	3	–	<i>C. cladospor.</i>	0	0
Yeasts (MF) 25 °C	<i>H. uvarum</i>	3	6	–	0	–	–	0	–
Culturable micro-organisms (total count), 3 days 22 °C	<i>P. fluorescens</i> (<i>E. aerogenes</i>) (<i>C. sakazakii.</i>) (<i>H. uvarum</i>)	0	0	<i>S. saprophyticus</i> (<i>E. coli</i>) (<i>C. freundii</i>)	0	0	<i>S. saprophyticus</i> (<i>E. coli</i>) (<i>K. pneumoniae</i>)	0	1

* In total 26 of 84 laboratories (31%) reported at least one deviating result

– Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

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

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

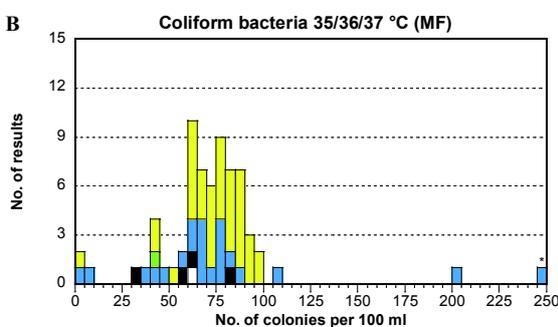
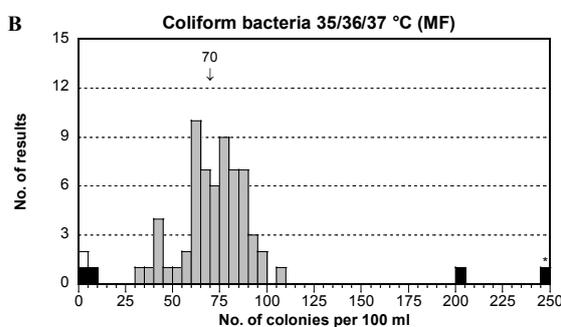
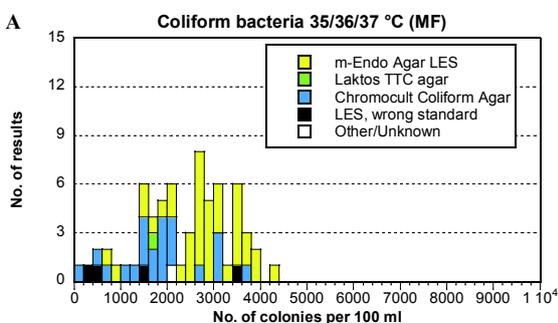
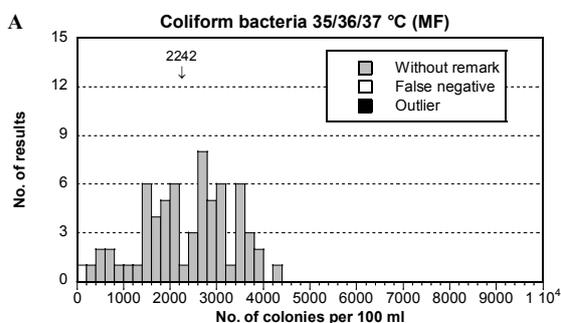
Coliform bacteria (MF)

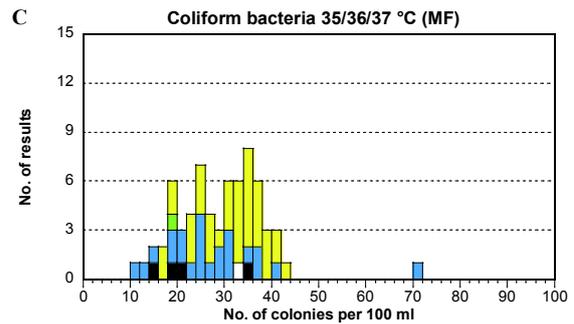
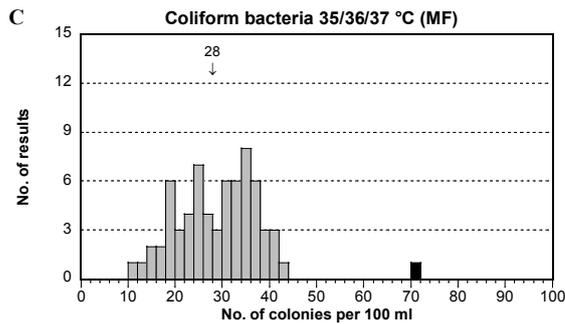
In a couple of cases m-Endo Agar LES (LES) has been used although not prescribed in the standard referred to (ISO 9308-1:2000 or ISO 9308-1:2014). These results have this time been placed in a separate group, "LES, wrong standard".

From the table it is clear that LES was used by far more laboratories than other media. The proportion that used CCA is now considerably higher than in previous rounds, while the use of LTTC has almost ended. This is reasonable since CCA has replaced LTTC in the latest edition of EN ISO 9308-1 from 2014.

There is an indication that LES gave a higher mean result compared to the other media for all samples. The implication is that CCA produced lower results with the coliform bacteria present in the samples. Despite the few results the average result for "LES, wrong standard" and the only one with LTTC are shown because they were the lowest ones.

Medium	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	68	65	2242	23	1	0	0	62	70	12	1	2	2	66	28	15	0	0	1
m-Endo Agar LES	39	38	2660	16	0	0	0	38	74	9	1	0	0	38	30	12	0	0	0
Laktos TTC Agar	1	1	1700	-	0	0	0	1	40	-	0	0	0	1	18	-	0	0	0
Chromocult C Agar	23	21	1828	22	1	0	0	18	67	12	0	2	2	22	25	16	0	0	1
LES, wrong standard	4	4	1119	-	0	0	0	4	58	-	0	0	0	4	21	19	0	0	0
Other/Unknown	1	1	2000	-	0	0	0	1	60	-	0	0	0	1	32	-	0	0	0





Mixture A

- No *E. coli* but two other strains of coliform bacteria were present in the mixture. Both these strains, *Enterobacter aerogenes* and *Cronobacter sakazakii*, appear usually with quite typical colonies at 37 °C, i.e. with metallic sheen on LES, a hue of yellow on LTTC and pink/red on CCA. The pink/red colony colour on CCA was, however, different for the two strains, as well as the colour zone around.
- There were some low results present that couldn't be identified as outliers. One result was even false negative. The average result with the rapid method (p.12) was higher, partly due to the fact that the two low results there were outliers.

Mixture B

- One strain of *Escherichia coli* and another coliform bacterium, *Citrobacter freundii*, were present. Both appeared with, for coliform bacteria, typical colonies on the MF media at 37 °C, a metallic sheen on LES and yellowish on LTTC. The colonies of *E. coli* were violet blue on CCA and those of *C. freundii* pink. The latter could be difficult to count as there was also a background flora with small pink colonies.
- Despite the background flora the distribution of the accepted results was good in general, but with an indication of a tail with low results. Both the 2 low and the 2 high outliers were from CCA. Otherwise, there was only one false negative result present.

Mixture C

- One strain of *Escherichia coli* was present together with a strain of *Klebsiella pneumoniae* as coliform bacteria. Both appeared with, for coliform bacteria, typical colonies on the MF media at 37 °C, a metallic sheen on LES and yellowish on LTTC. The colonies of *E. coli* were violet blue on CCA and those of *K. pneumoniae* pink to red. There was no problem with this analysis.
- One high outlier was present.

Suspected thermotolerant coliform bacteria (MF)

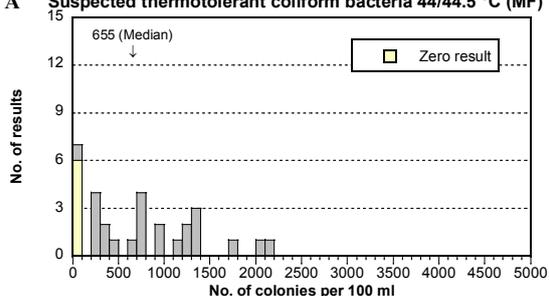
The two most used growth media were m-FC and LTTC. The incubation temperature is 44 or 44.5 °C. Because all old method details were removed before this round and that it this time was not mandatory to report method details for the parameter, very few laboratories reported method details. This made an evaluation meaningless and thus no grouping by method is given here.

There is never a performance assessment when suspected (not confirmed) colonies are evaluated and, therefore, no identification of outliers that are excluded in calculations. The *medians* are then more robust than the means and are given in the table and histograms. ***The parameter is not included in performance assessment.***

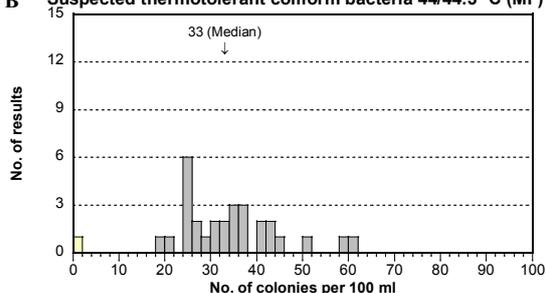
Standard, Method	Tot n	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
Total	30	30	655	–	–	–	30	33	–	–	–	30	23,5	–	–	–

Med = Median; used here instead of mean value

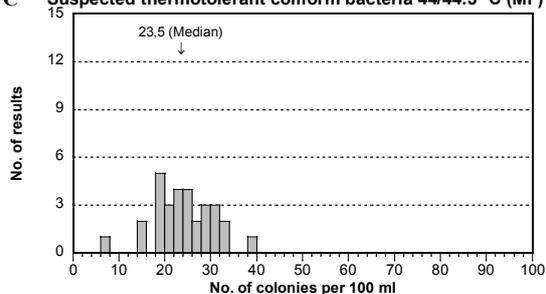
A Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



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



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



Mixture A

- There were no true thermotolerant coliform bacteria in the mixture. However, the two strains of coliform bacteria could grow as suspected thermotolerant coliform bacteria at 44 °C on m-FC agar, and probably also on LTTC agar that was not tested this time. The colonies of *E. aerogenes* were blue-grey on m-FC and those of *C. sakazakii* brownish grey surrounded by a light zone.
- The distribution of the 30 results was not as expected by a Poisson distribution. There was a substantial dislocation towards lower results, out of which 6 were zero results.
- The results all together indicate that many laboratories didn't identify the colonies as thermotolerant coliform bacteria, which is correct. But if colonies were present, they should probably have been judged as suspected thermotolerant coliform bacteria, that is the parameter asked for here. No confirmation, as test of gas production, should be taken into consideration but only colony growth and colour. Of the 9 laboratories that reported method information, only 2 have stated the use of 44.5 °C. One of these two laboratories has reported a zero result but there are no zero results reported by the 7 laboratories that stated the use of 44 °C. This makes it probable that incubation at 44.5 °C is one cause of zero results.

Mixture B

- The mixture comprised two coliform bacteria, of which the *E. coli* strain appears as a typical suspected thermotolerant coliform bacterium at 44 °C, meaning blue colonies on m-FC and yellow on LTTC. The strain is gas negative and could be excluded as a *confirmed* thermotolerant coliform bacterium when gas production is a criterion. The strain of *C. freundii* may sometimes appear with small blue colonies on m-FC when the temperature is as low as 43.5 °C.
- The distribution of the 30 results was good. There was no tail with low results, but only one zero result, indicating that gas production as a criterion was not used in case confirmed results were reported, with maybe one exception.

Mixture C

- One strain of *E. coli* was included together with a strain of *K. pneumoniae* as coliform bacteria. Both grow also as (suspected) thermotolerant coliform bacteria with typical colonies on the MF media, bluish on m-FC and yellow to dark yellow on LTTC at 44/44.5 °C.
- There was no problem with the analysis. No zero results were obtained.

Escherichia coli (MF)

E. coli is quantified after confirmation of colonies grown on the primary cultivation media LES and LTTC, incubated either at 36±2 °C or at 44/44.5 °C. Depending on method, either test of indole production or β-glucuronidase activity from oxidase negative presumptive strains is usually used. A violet to blue colony on CCA indicates positive β-glucuronidase activity and is reckoned as a confirmed *E. coli*.

The primary growth media CCA, LES as well as LTTC are used at 36±2 °C and LTTC or m-FC at 44/44.5 °C. All results are this time shown together but separated in groups based on used standard. For the standards from the Nordic countries (SS, SFS, NS) the majority of the results are from 36±2 °C but some also from 44/44.5 °C. The results are additionally grouped based on reported incubation temperature.

It is evident that the mean values in mixture B are the highest when it is clear that 36±2 °C has been used. CCA has given lower results and larger dispersion (CV) than LES (and the only LTTC result, which therefore also is given) at 36±2 °C in both mixture B and C. This has also been seen in earlier rounds but not as evident as this. It needs to be followed up also in coming rounds. When comparing standards, where results from 44/44.5 °C are also included, this difference is not equally evident.

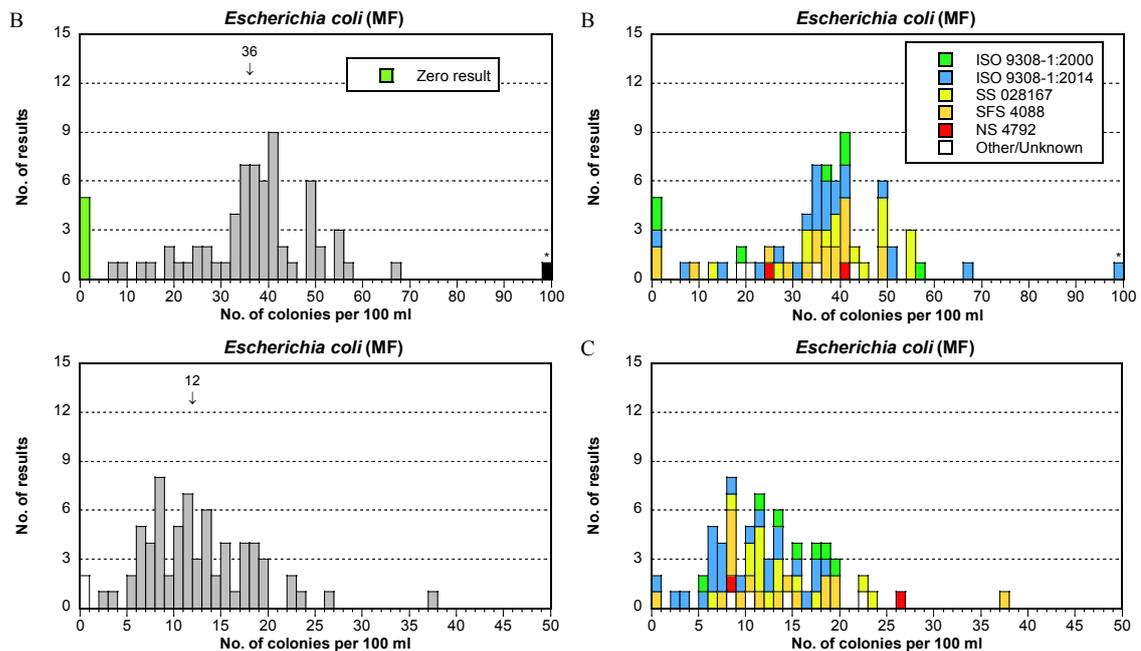
All results

Origin & Standard	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	70	67	0	-	1	-	-	63	36	18	5	0	1	67	12	24	2	0	0
<i>Colony origin</i>																			
36 ± 2 °C	41	40	0	-	1	-	-	37	38	16	3	0	1	39	13	23	2	0	0
44/44.5 °C	9	9	0	-	0	-	-	8	31	24	1	0	0	9	14	24	0	0	0
36 ± 2 & 44/44.5 °C	10	9	0	-	0	-	-	9	33	13	1	0	0	9	9	13	0	0	0
Other/Unknown	10	9	0	-	0	-	-	9	31	24	0	0	0	10	9	29	0	0	0
<i>Standard</i>																			
ISO 9308-1:2000	7	7	0	-	0	-	-	5	37	19	2	0	0	7	14	20	0	0	0
ISO 9308-1:2014	24	23	0	-	0	-	-	21	35	20	1	0	1	23	9	26	1	0	0
SS 028167	15	14	0	-	1	-	-	15	40	17	0	0	0	15	12	18	0	0	0
SFS 4088	18	17	0	-	0	-	-	16	35	17	2	0	0	16	13	25	1	0	0
NS 4792	2	2	0	-	0	-	-	2	-	-	0	0	0	2	-	-	0	0	0
Other/Unknown	4	4	0	-	0	-	-	4	-	-	0	0	0	4	-	-	1	0	0

Results from the analysis of coliform bacteria MF

Medium	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	40[#]	39	0	-	1	-	-	36	39	15	3	0	1	38	13	23	2	0	0
m-Endo Agar LES	27	26	0	-	1	-	-	25	40	14	3	0	0	26	14	22	1	0	0
Lactose TTC Agar	1	1	0	-	0	-	-	1	40	-	0	0	0	1	18	-	0	0	0
Chromocult C Agar	12	12	0	-	0	-	-	10	36	18	1	0	1	11	10	24	1	0	0
Other/Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-

Compare table above – one more laboratory performed the analysis of *E. coli* than of coliform bacteria



Mixture A

- No *E. coli* was included but two other suspected thermotolerant coliform bacteria. One false positive result was reported.

Mixture B

- A gas negative strain of *E. coli* was included together with another coliform bacterium, *C. freundii*. The colony appearance for *E. coli* is typical on the various media used. When a too low temperature is used, small atypical colonies of *C. freundii* may appear on the media for thermotolerant coliform bacteria (44/44.5 °C). On other media, the appearance of *C. freundii* is typical for coliform bacteria. Because of that, confirmation of colonies from LES and LTTC is needed to identify *E. coli*.
- The strain of *E. coli* is positive when testing for indole production and/or β -glucuronidase activity. When gas production is an additional criterion the outcome of the confirmation will be negative since the strain is gas negative.
- Five false negative results were reported together with 1 high outlier. A tail of further low results is seen in the histogram.

Mixture C

- One typical strain of *E. coli* was present together with another thermotolerant coliform bacterium, *K. pneumoniae*. The distribution of the results was good even though the dispersion was of average size.
- Two false negative results were present.
- As mentioned, CCA gave lower results and larger dispersion than LES at 36±2 °C
- In principle the same average was obtained as with the rapid methods (p. 13).

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

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

The differences were small when the numbers of wells on the trays as well as different incubation times were compared. Therefore, such grouping is not shown.

A difference based on the maximum incubation length could be seen for coliform bacteria in mixture A. The maximum 22 hours gave somewhat higher average results than the maximum 20 hours. Tendencies to similar behaviour are seen also for mixture B and C. However, no such tendency was at all seen for *E. coli*.

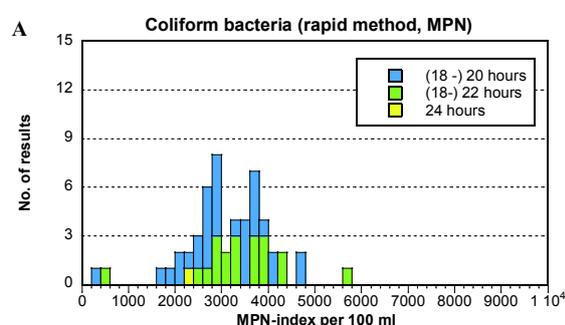
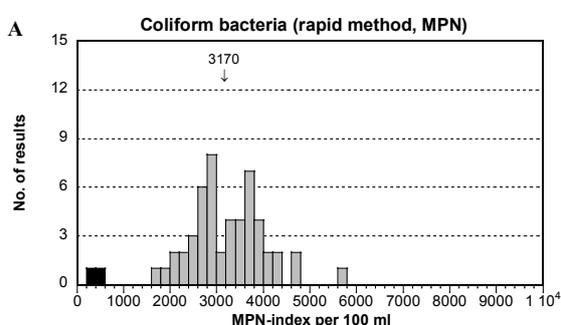
In no case there seem to have been any problem with interpretation of the results.

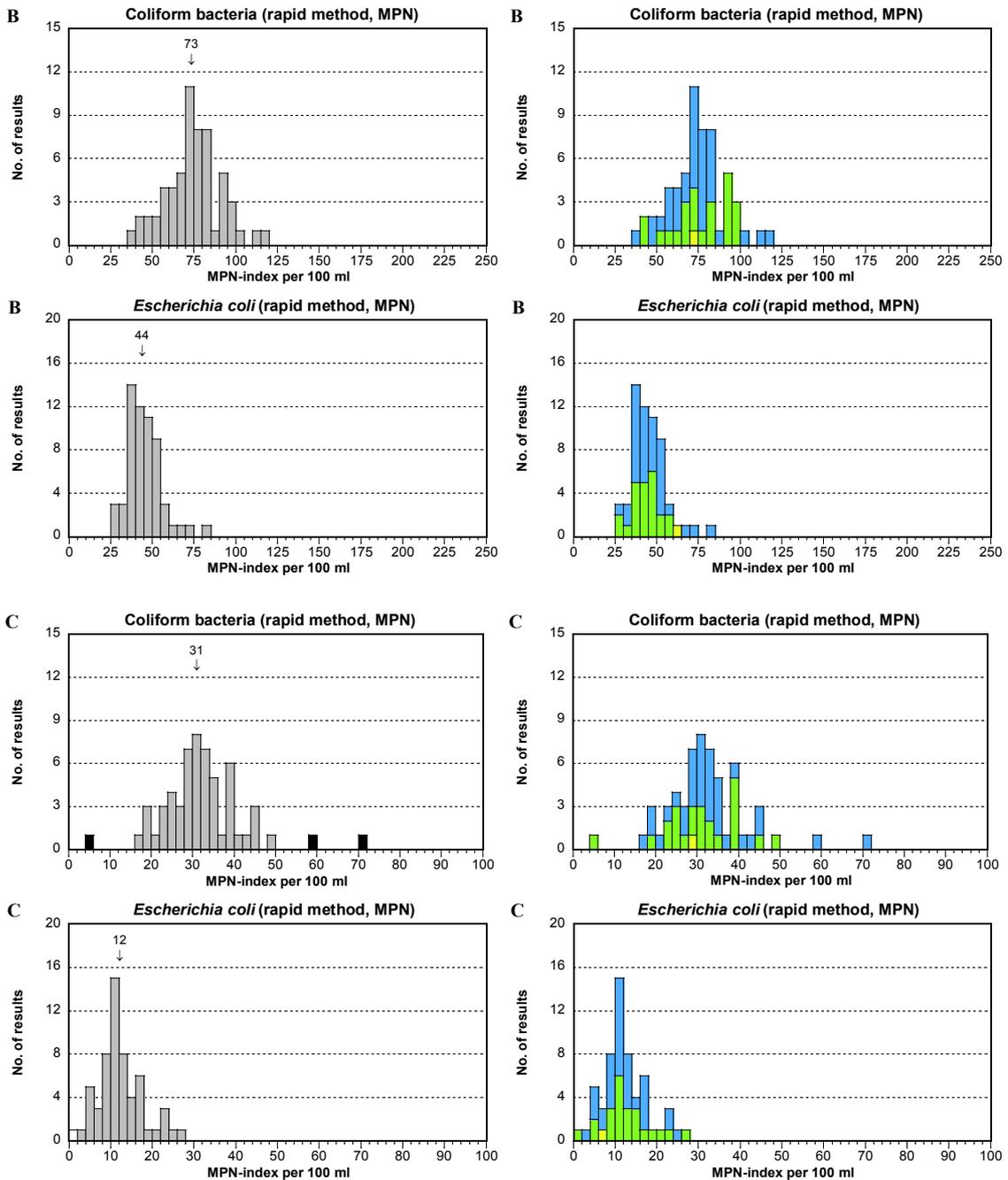
Coliform bacteria, Rapid method with MPN

Incubation time	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	60	60	0	–	0	–	–	58	321	21	0	0	0	60	34	11	0	0	0
(18-) 20 hours	35	31	3028	13	0	1	0	35	72	11	0	0	0	32	30	12	0	0	2
(18-) 22 hours	23	19	3460	10	0	1	0	23	74	12	0	0	0	22	31	12	0	1	0
(23-) 24 hours	1	1	–	–	0	0	0	1	–	–	0	0	0	1	–	–	0	0	0

E. coli, Rapid method with MPN

Incubation time	N	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	60	60	0	–	0	–	–	59	0	–	0	–	–	59	15	18	1	0	0
(18-) 20 hours	35	34	0	–	0	–	–	35	44	12	0	0	0	34	11	23	0	0	0
(18-) 22 hours	23	23	0	–	0	–	–	23	42	10	0	0	0	22	12	21	1	0	0
(23-) 24 hours	1	1	0	–	0	–	–	1	–	–	0	0	0	1	–	–	0	0	0





Mixture A

- Two different coliform bacteria but no *E. coli* was included. The histogram seems to have two peaks. The reason is not clear but it indicates that the outcome of at least one of the coliform bacteria, *E. aerogenes* and *C. sakazakii*, may have been interpreted differently among the laboratories. The question is whether some wells was yellow or not, which for some strains may be time dependent. A standard tray, a comparator, to compare with is then necessary. Probably, a different

interpretation is reflected in the differences among the results after a maximum of 20 and 22 hours, respectively.

- The dispersion (CV) was yet small and the only deviating results were 2 low outliers.
- The average result is somewhat higher than compared to the MF method (p. 6).

Mixture B

- In this mixture were the coliform bacteria *E. coli* and *C. freundii* present. Both of them possess β -galactosidase (ONPG positive) and are detected as coliform bacteria. Only *E. coli* possesses β -glucuronidase and is detected as *E. coli*.
- The distributions of the results were good and the dispersions (CV) were small for both coliform bacteria and *E. coli*. No deviating results were present.
- The average results are somewhat higher compared to the MF methods.

Mixture C

- The strains of *E. coli* and *K. pneumoniae* grow in the medium and have the enzyme β -galactosidase. Therefore, they are detected as coliform bacteria by methods based on this enzyme (ONPG positive) e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate.
- One low outlier and 2 high outliers were reported for coliform bacteria.
- Only the strain of *E. coli* has the enzyme β -glucuronidase and is detected as *E. coli*. One false negative result was obtained in the analysis of *E. coli*.
- The distributions of the results were good and the dispersion (CV) was small for coliform bacteria. The dispersion for *E. coli* was medium, mainly because the average concentration was low and thus the relative dispersion large.
- The average for coliform bacteria is somewhat higher for the rapid methods than for the MF methods and about the same for *E. coli*.

Presumptive and confirmed *Clostridium perfringens* (MF)

The analysis of *Clostridium perfringens* is performed differently in different countries and laboratories, because no international standard was stated as reference method in the European Drinking Water Directive from 1998 (4). The parameter to be analysed is spores and vegetative cells of *C. perfringens*. In Sweden the presumptive *C. perfringens* are reported, why this parameter is presented separately.

There is an interim method explicitly described in the Drinking water directive from 1998 (4), the use of m-CP Agar incubated at 44 °C. The method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*. Due to the hesitation in many countries to use this method, the use of a standard still under process (ISO/CD 6461-2:2002-12-20, CD = Committee Draft) based on TSC agar was accepted as an alternative by the responsible group under the EU Commission, until a finished standard was available. Adjustments in the draft approved in ISO meetings during the standardization process have been included in the instructions for proficiency testing rounds, e.g. colour on colonies to be counted.

The standard ISO 14189 was finished in November 2013 and the identical EN ISO 14189 and its national editions were finished in 2016. The standard is basically equivalent to the CD version from 2002 after adjustments, but has a much more simplified confirmation step. In the new standard, isolated colonies are only tested for activity of the enzyme acid phosphatase. The new standard was in October 2015 included in the revised annexes to the directive text and should be taken into usage no later than in October 2017 within EU, after being implemented in the national legislations. The CD version will be invalid for use in official drinking water monitoring after that date.

Two laboratories have reported "Other" as reference than the three methods mentioned above, without stating anything else. One more laboratory reporting "Other" has referred to the CD version but analysed spores only. The results from these three laboratories are included in the group Other/Unknown.

To be able to compare here as well as against previous rounds, mean and dispersion for m-CP agar is given for presumptive *C. perfringens* despite few results. The medians are furthermore much lower than the means for mixture B and C, 294 and 66 cfu/100 ml, respectively. Both for mixture B and C gave m-CP agar lower recovery compared to TSC agar (which is the medium in the two other method references) both for presumptive *C. perfringens* and *C. perfringens*. For presumptive *C. perfringens* only 4 laboratories have used m-CP agar. Yet, for the discussion the dispersion is given for them. The histograms show clearly that the results of m-CP Agar are mainly in the lower end of the results.

In mixture C with 11 results for *C. perfringens* with m-CP agar, that method has the largest dispersion (CV). The same tendency is seen in the other mixtures as well, though there were only 4 results present. The dispersion was very large for presumptive *C. perfringens* (here *C. bifermentans*) for all methods in mixture B. The results mentioned may not be generally valid but have also previously been seen for those strains of *C. bifermentans* and *C. perfringens* used in the mixtures.

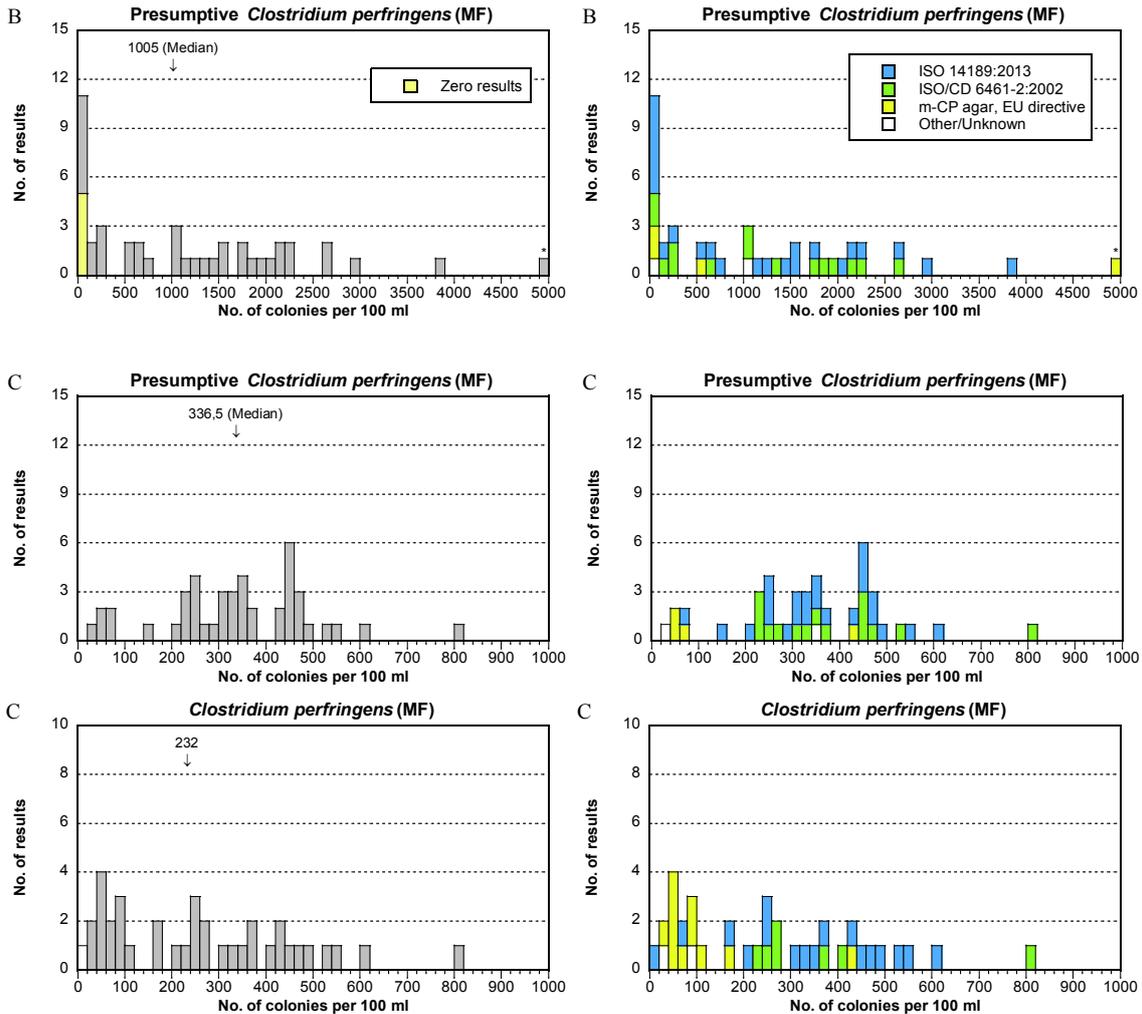
Presumptive *Clostridium perfringens* MF

Standard/Method	N	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
Total	61	43	0	-	1	-	44	790	69	0	0	0	44	314	27	0	0	0
ISO 14189:2013	29	22	0	-	1	-	23	783	70	0	0	0	23	339	21	0	0	0
ISO/CD 6461-2:2002	18	15	0	-	0	-	15	919	51	0	0	0	15	367	19	0	0	0
m-CP agar, EU-direct.	11	4	0	-	0	-	4	700	127	0	0	0	4	122	59	0	0	0
Other/Unknown	3	2	0	-	0	-	2	-	-	0	0	0	2	-	-	0	0	0

Clostridium perfringens MF

Standard/Method	N	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
Total	61	36	0	-	0	-	35	0	-	1	-	-	36	232	40	1	0	0
ISO 14189:2013	29	17	0	-	0	-	17	0	-	0	-	-	16	341	24	1	0	0
ISO/CD 6461-2:2002	18	6	0	-	0	-	5	0	-	1	-	-	7	352	24	0	0	0
m-CP agar, EU-direct.	11	11	0	-	0	-	11	0	-	0	-	-	11	94	43	0	0	0
Other/Unknown	3	2	0	-	0	-	2	0	-	0	-	-	2	-	-	0	0	0

N is here the number of laboratories that have reported results either for presumptive *C. perfringens*, *C. perfringens* or both.



Mixture A

- No presumptive *C. perfringens* was included. Yet, one false positive result was present for presumptive *C. perfringens*.

Mixture B

- No *C. perfringens* was included but a strain of *C. bifermentans*. The strain appeared on TSC with black to almost transparent colonies. Confirmation reveals that they are not from *C. perfringens*.
- The distribution of the results is bad. The dispersion (CV) was very large in the presumptive test with the implication that no outliers could be identified. Five zero results were obtained, out of which one was from m-CP agar.
- In the analyses of *C. perfringens* one false positive results was present.

Mixture C

- A strain of *C. perfringens* was included. The colour of the colonies on TSC could vary from pale grey-brown to completely black depending on the condition and reduction potential of the medium.
- No deviating results could be identified from the presumptive test but one false positive result was present for *C. perfringens*.
- The distribution of the results was quite scattered but still quite good for presumptive *C. perfringens*. In contrast, there was a large over-representation of low results for *C. perfringens*. The many low results make it difficult to identify deviating results. The dispersion as CV was large for the m-CP agar method and of medium size for the other methods.

Moulds and yeasts (MF)

Out of the 36 laboratories that analysed moulds and yeasts, 30 reported that they used the Swedish standard SS 028192. Besides Sweden it is used in Denmark and also in Finland and Norway under their own national designations SFS 5507 and NS 4716, respectively.

Various names, some appropriate and other probably inappropriate, were reported for the media linked to the use of SS 028192. These are "Cooke Rose Bengal Agar base", "Rose Bengal Agar base", "Rose Bengal Agar", "Rose Bengal Chloramphenicol Agar" (RBC) and "Dichloran Rose Bengal Chloramphenicol Agar" (DRBC). According to the standard, dichloran should not be an ingredient (and thus DRBC should not be used) but instead Rose Bengal and the two stronger inhibitory substances chlortetracycline and chloramphenicol are authorized. Both of them are usually used by the 22 Swedish laboratories. Here is shown what the laboratories have really stated, and a separation is made for those that have used any form of Rose Bengal Agar (RBC Agar) and those stating "Dichloran Rose Bengal Chloramphenicol" (DRBC Agar, Water).

One Norwegian laboratory used NMKL 98:2005, modified together with DRBC. This comprise the group DRBC A "Food" in the tables. Four Finnish laboratories used Malt Extract Agar, out of which one in conjunction with NMKL 98:2005 and the other with other non-water methods. They are placed in a separate group, ME Agar. The 7 laboratories from various countries that have used DRBC together with SS 028182 or SFS 5507, or in one case "Standard methods" (5), is placed in the group DRBC A "Water". No laboratory is placed in the group Other/Unknown. For some groups the numbers of results are so few that it is not meaningful to discuss any differences. Yet, mean values are given as comparison for some of these groups.

Moulds MF

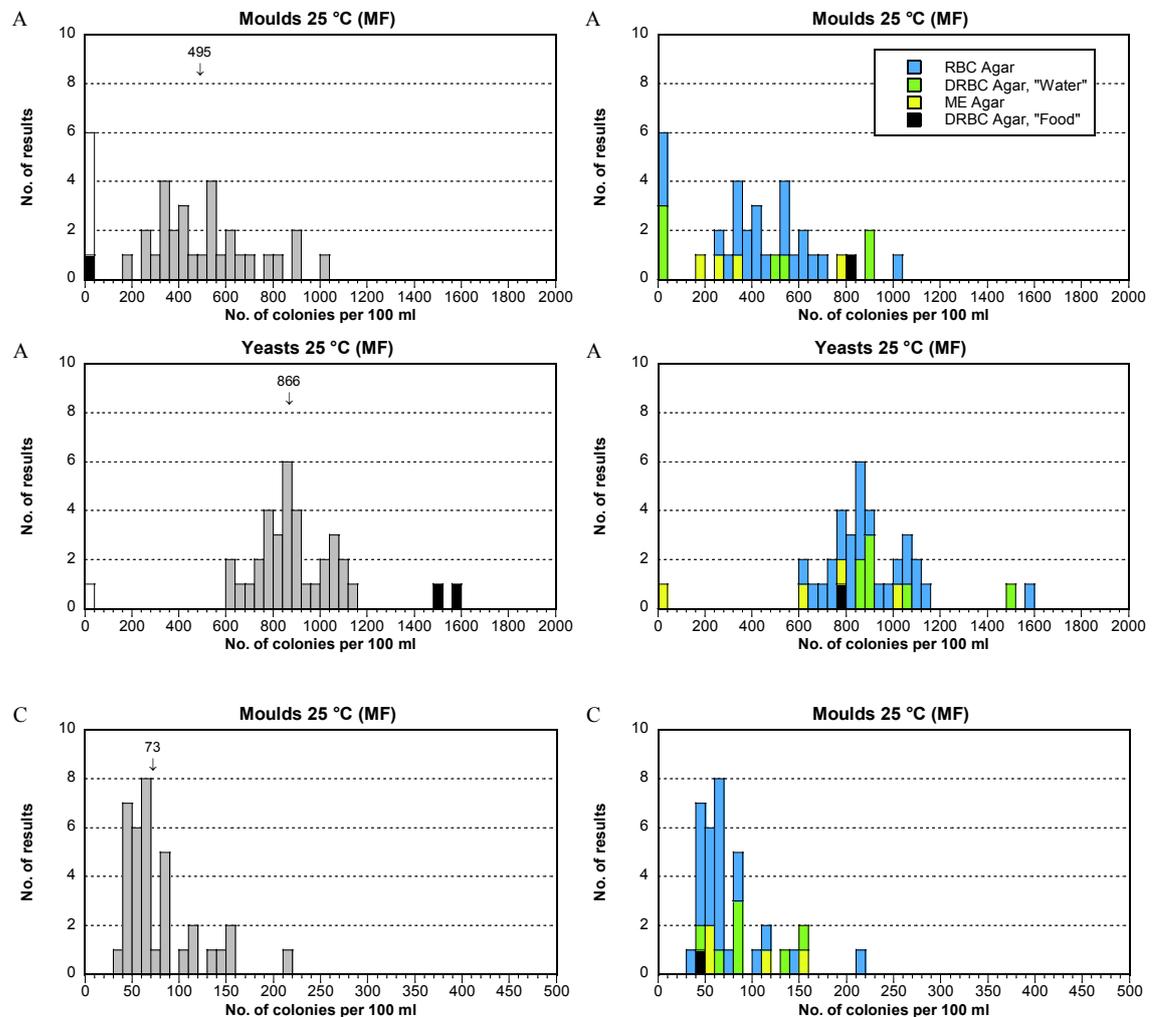
Standard/Method	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	36	29	495	21	5	1	0	35	0	–	1	–	–	36	73	23	0	0	0
RBC A(gar)	24	20	473	17	2	1	0	23	0	–	1	–	–	24	68	23	0	0	0
DRBC A "Water"	7	4	700*	16	3	0	0	7	0	–	0	–	–	7	88	21	0	0	0
ME Agar	4	4	365*	–	0	0	0	4	0	–	0	–	–	4	90*	–	0	0	0
DRBC A "Food"	1	1	–	–	0	0	0	1	0	–	0	–	–	1	–	–	0	0	0
Other/Unknown	0	0	–	–	0	0	0	0	–	–	0	–	–	0	–	–	0	0	0

Yeasts MF

Standard/Method	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	36	33	866	8	1	0	2	36	0	–	0	–	–	36	0	–	0	–	–
RBC A(gar)	24	23	871	8	0	0	1	24	0	–	0	–	–	24	0	–	0	–	–
DRBC A "Water"	7	6	908	4	0	0	1	7	0	–	0	–	–	7	0	–	0	–	–
ME Agar	4	3	780*	–	1	0	0	4	0	–	0	–	–	4	0	–	0	–	–
DRBC A "Food"	1	1	–	–	0	0	0	1	0	–	0	–	–	1	0	–	0	–	–
Other/Unknown	0	0	–	–	0	0	0	0	–	–	0	–	–	0	–	–	0	–	–

* Mean values are given despite few results; the median values are lower in all three cases pertaining ME Agar

In the three cases with numerical results the average is somewhat higher for both moulds and yeasts with DRBC Agar "Water" compared with RBC Agar. Although there are only 4 values, the mean and median indicate that ME Agar gave lower results in mixture A than the two groups where a selective water method were used.



Mixture A

- The mould *Phialophora malorum* and the yeast *Hanseniaspora uvarum* were included. The yeast colonies occurred in twice the number of mould colonies. The distributions of the results except the deviating ones were quite good. The dispersion as CV was medium for moulds but very small for yeasts.
- Five false negative results and one low outlier were present for the moulds.
- One false negative result and 2 high outliers were present for the yeasts.
- There seemed to be no major problem with the analyses. The reason to the false negative mould results is probably that the quite late sporulating mould colonies were taken for yeast colonies. This is supported by the fact that the laboratories with zero results for moulds have reported high results for yeasts. The lowest of

these results is 900 cfu/100 ml and the rest are above 1000 cfu/100 ml, out of which one is a high outlier. In the histogram for yeasts there is also a secondary peak around approximately 1100 cfu/100 ml.

Mixture B

- No moulds nor yeasts were included. Yet, a false positive result was reported for moulds.

Mixture C

- No yeasts were present but the mould *Cladosporium cladosporoides* was included. The distribution and dispersion of moulds were quite good with a medium dispersion (CV).
- No deviating result was present.
- There seemed to be no problem with the analysis.

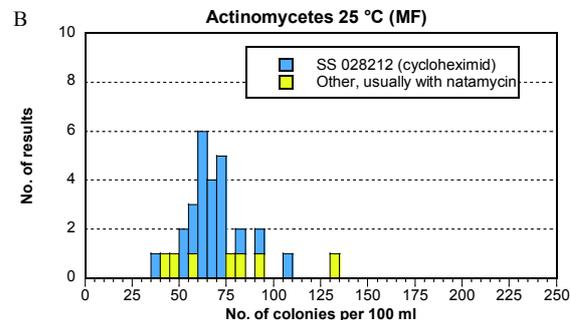
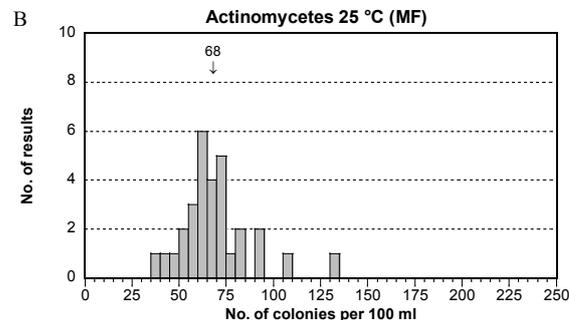
Actinomycetes (MF)

The analysis of actinomycetes is included because it is among the methods that should regularly be used according to the Swedish regulations. Therefore, it is mainly Swedish laboratories that performed the analysis according to the Swedish standard for actinomycetes in water, SS 028212 (1994). Seven Finnish laboratories that have performed the analysis based on other methods are placed in the group Other. Five of these have stated that they used natamycin as the selective substance instead of cycloheximide. Not even the other 2 laboratories have used cycloheximide but didn't state what they used. The base agar medium varies also within the group Other but is in all cases different from Actinomycete Isolation Agar (ACTA) that is the base medium in the Swedish standard.

The average for the group Other in mixture B is somewhat higher, while the dispersion (CV) is twice as large, compared to the group ACTA. This pertains to the strain and sample included here but cannot be considered to be generally valid.

All results

Medium/Standard	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	30	30	0	-	0	-	30	68	13	0	0	0	29	0	-	1	-	-	-
ACTA (SS 028212)	23	23	0	-	0	-	23	66	10	0	0	0	22	0	-	1	-	-	
Other	7	7	0	-	0	-	7	73	20	0	0	0	7	0	-	0	-	-	



Mixture A and C

- These mixtures contained no actinomycetes. One false positive result was reported for mixture C.

Mixtures B

- One actinomycete within the group *Streptomyces sp.* was included. The distribution of the results was good and the dispersion small.
- The analyses seemed to be without problem. No deviating results were present.

Culturable microorganisms 22 °C, 3 days

Seventy nine of the 82 laboratories performing the analysis reported EN ISO 6222:1999 as method, which prescribes the use of Yeast extract Agar. Five laboratories used Plate Count Agar instead, but have simultaneously stated the use of EN ISO 6222:1999. Two laboratories used Yeast extract agar in conjunction with "Standard methods" (5), one of which stated spread plating instead of pour plating. The majority of the laboratories have claimed counting both bacteria colonies as well as fungal colonies while 13 report that they don't count fungi. Four others tell that they include yeasts when counting but not moulds.

Since all except 3 laboratories refer to EN ISO 6222:1999, differences among method variants are relevant to discuss only for these. Results are shown for culture media and magnification of reading.

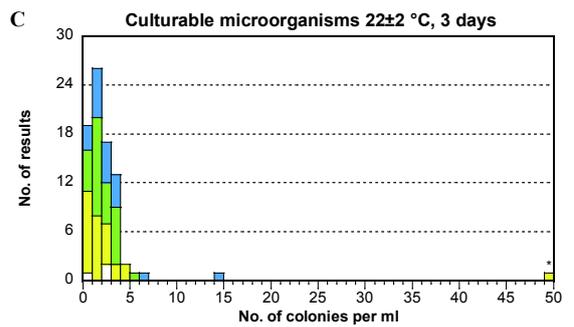
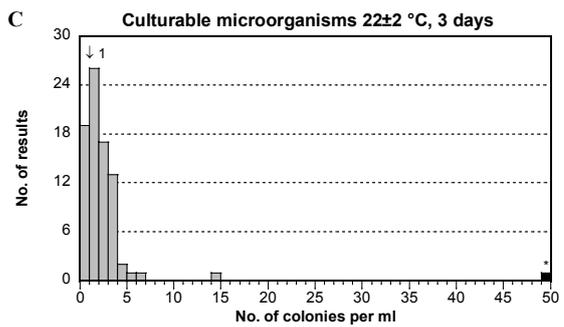
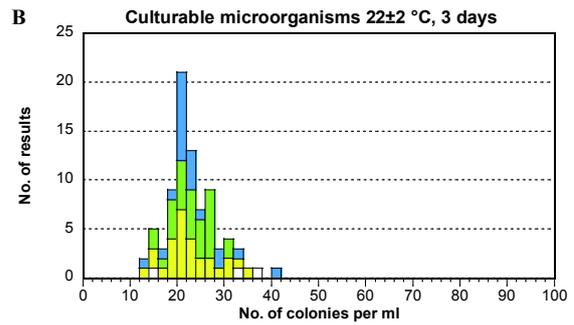
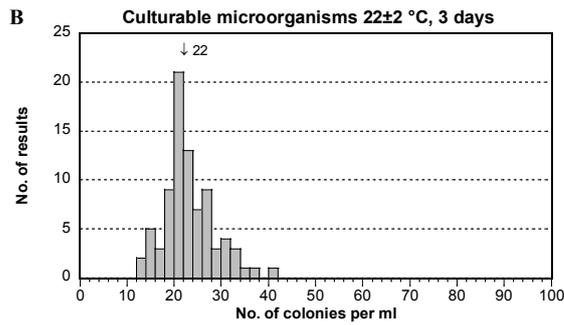
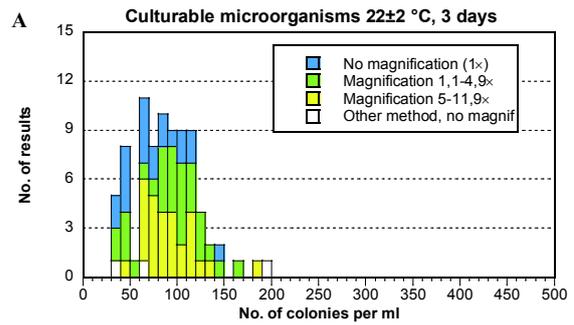
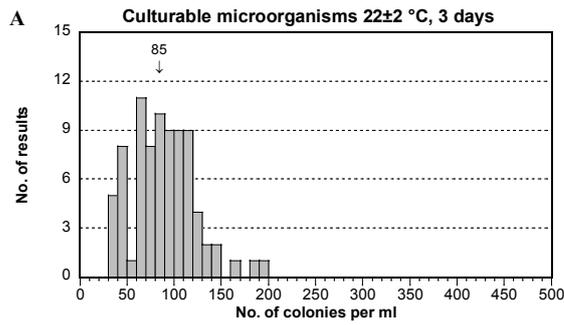
It is difficult to find any method difference that is consistent between the mixtures. In mixture C the concentration is too low to discern any possible differences. In mixture A, Plat Count Agar seems to result in higher average than Yeast extract Agar. But with only 5 results and medium dispersion that difference is questionable. It seems more certain that reading without magnification has given lower results than with magnification in mixture A. However, this is not the case for mixture B. Such differences are possible as some samples may give very small colonies difficult to count, while others don't.

The distributions were good for all mixtures. The dispersion as CV was, however, very large in mixture C due to the very low concentration of organisms, about 1 cfu/ml. In the other two mixtures the dispersions were small.

Group of results	N	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	82	81	85	19	0	0	0	82	22	12	0	0	0	81	1	70	0	0	1
EN ISO 6222	79	78	85	18	0	0	0	79	22	11	0	0	0	77	1	68	0	0	1
<u>Medium</u>																			
Yeast extract Agar	74	73	83	16	0	0	0	74	22	11	0	0	0	72	1	69	0	0	1
Plate Count Agar	5	5	116	27	0	0	0	5	20	8	0	0	0	5	1	58	0	0	0
Other/Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<u>Magnification</u>																			
None	21	20	72	20	0	0	0	21	22	12	0	0	0	20	2	65	0	0	0
1,1-4,9×	30	30	88	19	0	0	0	30	22	9	0	0	0	30	1	55	0	0	0
5-11,9×	28	28	90	15	0	0	0	28	22	12	0	0	0	27	1	85	0	0	1
> 12×	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Other method	3	3	-	-	0	0	0	3	-	-	0	0	0	3	-	-	0	0	0

Mixture A

- It is mainly colonies of *Pseudomonas fluorescens* that is visible but also the coliform bacteria and the yeasts may appear with individual colonies.
- The distribution of the results was good and no deviating results were present.



Mixture B

- The colonies are mainly made up by the strain of *Staphylococcus saprophyticus* but individual colonies of the coliform bacteria and the actinomycete will also appear.
- The distribution of the results was good with no deviating ones.

Mixture C

- The number of colonies appearing was very low, about 1 cfu/ml. What mainly grow are colonies of *Staphylococcus saprophyticus*. Also individual colonies of coliform bacteria may sometimes show up.
- Due to the very low average, also a zero result is appropriate and acceptable. The distribution was, however, good in general with only 1 high outlier.

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. These values are highlighted with bold text on yellow background in annex A. The limit values for lowest and highest accepted results are given for each 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 performance can broadly be assessed by the numbers of false results and outliers given beneath the box plots.

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

A number of laboratories have several deviating results. When whole samples seem to have been mixed up, the corresponding sample numbers are hatched in annex A. This time no laboratory seems to have mixed up vials and even not sample/results for individual analyses. A couple of laboratories may have performed incorrect calculations from their colony readings to the final concentrations.

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

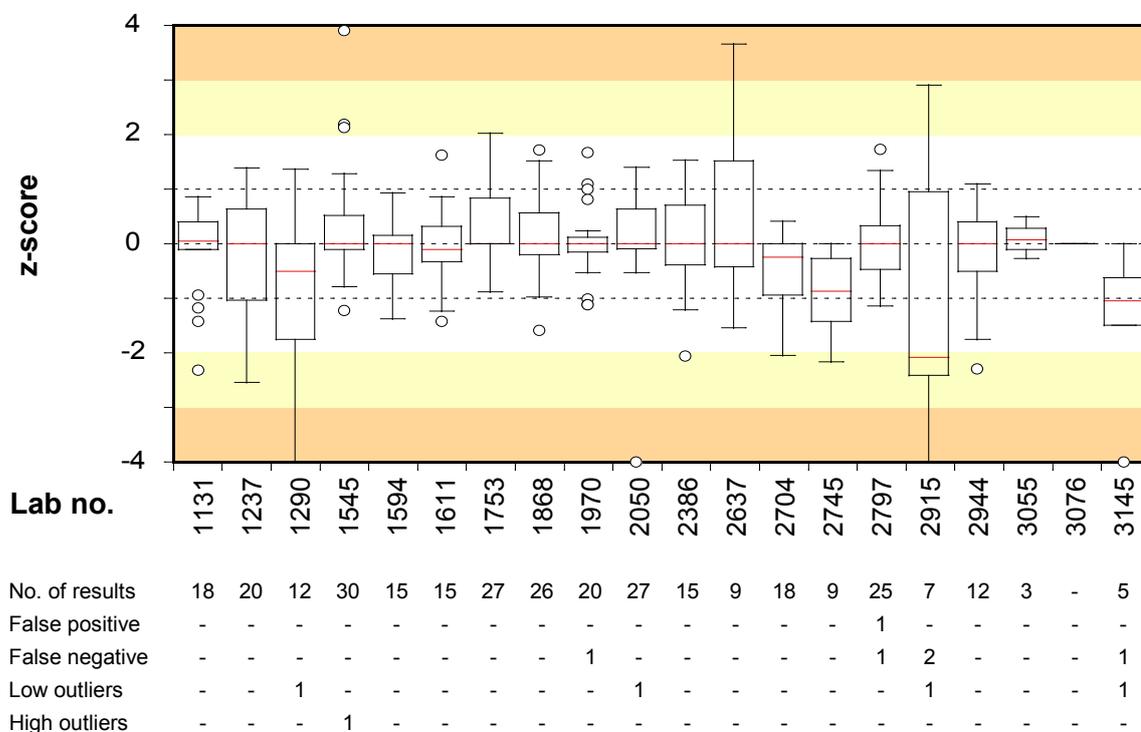
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are reported in annex B but not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control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

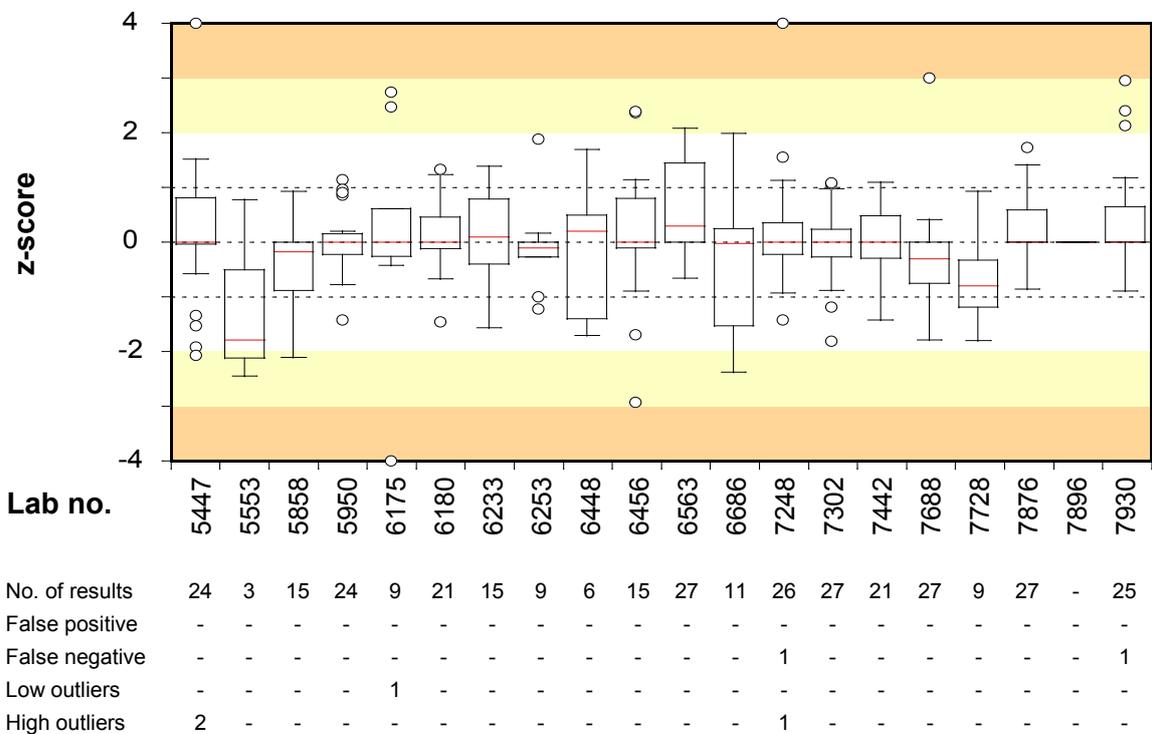
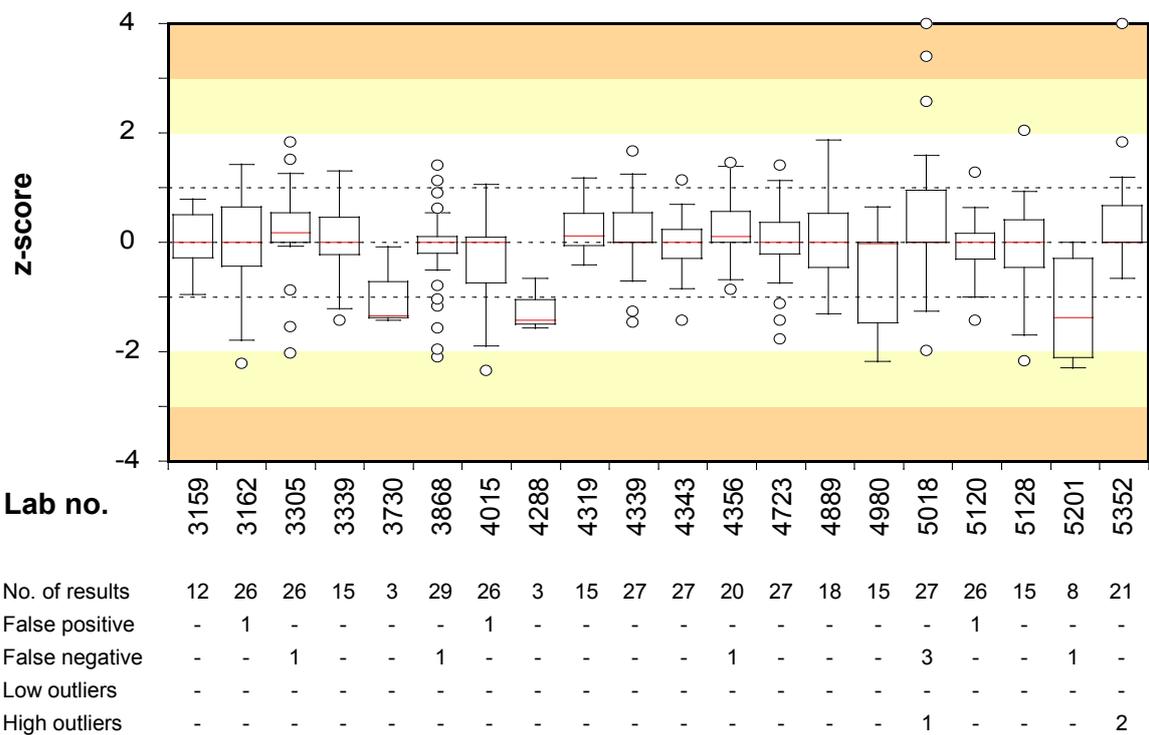
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

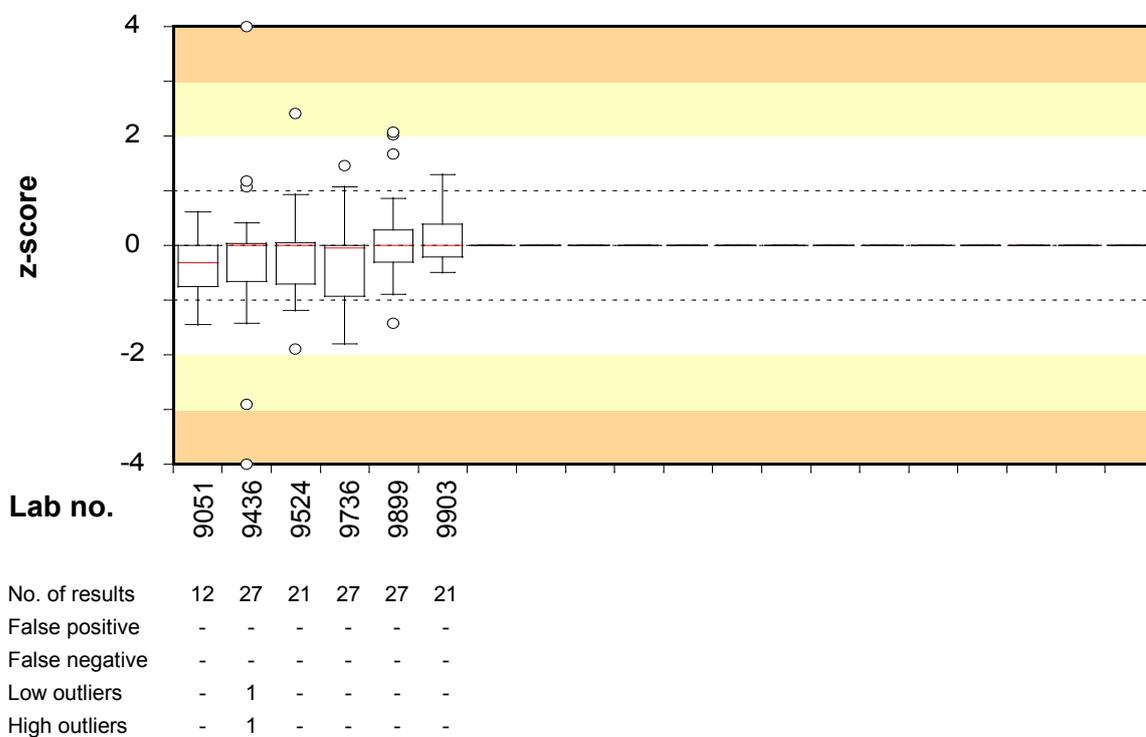
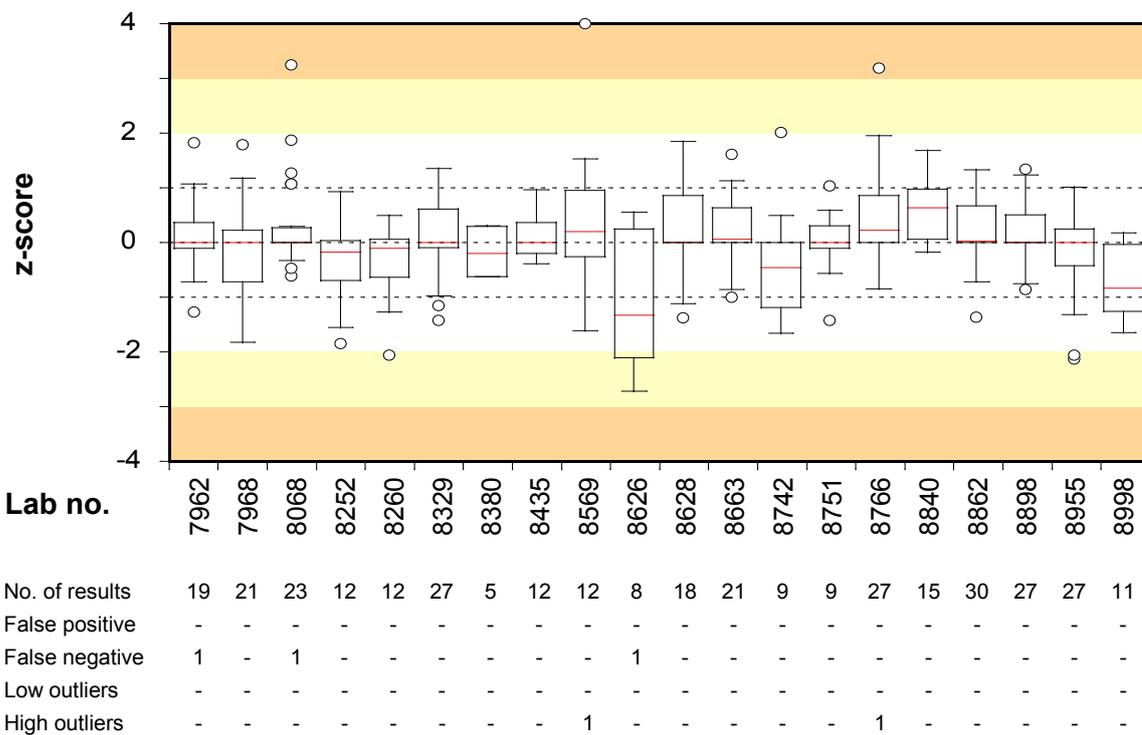
Box plots and numbers of deviating results for each participating laboratory

- z-scores are calculated from the formula $z = (x - mv) / s$ (see annex A).
- A correct result "zero" will get $z = 0$ when there is no target organism present.
- False results do not generate z-scores and are not included in 'No. of results'.
- The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as the rest of the results.
- Z-scores $> +4$ and < -4 have in the plots been set to $+4$ and -4 , respectively.
- 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 to a certain degree deviating* from the rest.
- The background is divided into coloured fields to simplify localization of the laboratory results.

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







Test material, quality controls and processing of data

Description of the test material

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

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

Table 2 *Microorganisms present in the mixtures*

Mixture ¹	Microorganisms	Strain collection no.		cfu/100 ml ²
		SLV (own)	Reference ³	
A	<i>Enterobacter aerogenes</i>	099	ATCC 13 048	2000
	<i>Cronobacter sakazakii</i>	419	–	1300
	<i>Hanseniasspora uvarum</i>	555	CF SQE 77	1000
	<i>Phialophora malorum</i>	545	–	800
	<i>Pseudomonas fluorescens</i>	535	CCUG 45106	120 *
B	<i>Escherichia coli</i>	532	CCUG 48891	23
	<i>Citrobacter freundii</i>	091	CCUG 43597	22
	<i>Clostridium bifermentans</i>	009	CCUG 43592	1000
	<i>Streptomyces sp.</i>	548	–	320
	<i>Staphylococcus saprophyticus</i>	013	CCUG 45100	20 *
C	<i>Escherichia coli</i>	084	–	14
	<i>Klebsiella pneumoniae</i>	186	CCUG 45102	18
	<i>Clostridium perfringens</i>	442	CCUG 43593	330
	<i>Cladosporium cladosporoides</i>	488	CBS 812.96	110
	<i>Staphylococcus saprophyticus</i>	013	CCUG 45100	1 *

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 and 2 of table 3

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

3 Origin or typing collection no.; ATCC: American Type Culture Collection; CCUG: Culture Collection University of Gothenburg, Sweden; CBS: Centraalbureau voor Schimmelcultures, Utrecht, Holland; – only in our own culture collection

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 24, 11 and 15 dispensed aliquots in vials, for mixture A, B and C, respectively. The largest differences between vials were 9, 4 and 9 mg, respectively, in the mixtures. The largest accepted difference is 15 mg (3%).

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

Analysis parameter <i>Method standard for analysis</i>	Mixture								
	A ¹			B ¹			C ²		
	cfu	I_2	T	cfu	I_2	T	cfu	I_2	T
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	33 ^a	2.0	1.7	45 ^c	0.3	1.2	32	2.4	1.7
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	9 ^a	2.0 ^d	2.7	38	1.5	1.5	30	2.0	1.8
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	0 ^a	–	–	23 ^c	0.6	1.4	14	1.4	1.9
Presumptive <i>Clostridium perfringens</i> (MF) <i>TSC Agar according to ISO/CD 6461-2:2002</i>	–	–	–	10 ^a	1.1	2.0	33 ^b	1.6	1.5
Moulds (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	8 ^a	0.5	1.6	–	–	–	11 ^b	1.0	1.8
Yeasts (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	10 ^a	0.7	1.6	–	–	–	–	–	–
Actinomycetes (MF) <i>Actinomycete Isolation Agar with cycloheximide according to SS 028212</i>	–	–	–	32 ^c	1.0	1.4	–	–	–
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	162	1.8	1.2	20	0.3	1.3	1	0.7	4.7

1 10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, analysed 20 and 19 weeks ahead of the testing round for the mixtures A and B, respectively

2 5 vials analysed in duplicate (stability check), 2 weeks ahead of the testing round

a Determined for the volume 1 ml

b Determined for the volume 10 ml

c Determined for the volume 50 ml

d The value is <2.0 but is rounded up to 2.0

– No target organism and thus no analysis

Table 3 presents the results from the organizer in the form of concentration means (cfu) and the measures (I_2 and T; see reference 1) used to assess homogeneity from duplicate analyses of 10 vials from each mixture the first time a mixture is used or duplicate analyses from 5 vials in a stability check when a mixture is used a second time. The results relate to the volume that was used for counting the colonies. The criterion used for a mixture to be considered homogenous is that I_2 and T are *not simultaneously* higher than 2. According to that criterion, all mixtures were homogeneous regarding the parameters that were about to be analysed.

Processing of numerical results

Most histograms have "tails" in either or both directions, due to values that do not belong to a normal distribution. 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 of mean values and measures of distribution.

The coefficient of variation (CV) for square root transformed results is given as a measure of dispersion. When the dispersion is <10% it is regarded as very small, 10–20% as small, 20–30 % as medium, 30–40% as large and >40% as very large.

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

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

References

1. Anonymous 2015. Scheme protocol, Microbiology, Drinking water & Food, 4th ed. National Food Agency, Sweden.
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4. Anonymous 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Official Journal of the European Communities.* 5.12.98, L 330/32-54 (*national translations available*).
5. Standard Methods for the Examination of Water and Wastewater, <http://www.standardmethods.org/>
6. Anonymous 2015. Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption. *Official Journal of the European Union.* 7.10.2015, L 260/6-17 (*national translations available*).

Annex A Results of the participants. Susp. = suspected on membrane filter before confirmation. Results given as <1, <2, <10 and <100 are treated as zero. The fields with other results given as < 'value' and results given as > 'value' are **yellow**, and those results are not included in calculations or evaluations, as are also not results in **shaded columns**. **Empty hatched fields** indicate that the result has been deleted due to misunderstanding of instructions or use of improper method. 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 are excluded, as well as other outliers, in the summarizing

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 1 3	2600	70	34	2600	70	34	-	-	-	0	37	17	3654	71	23	0	45	12
1237	1 2 3	-	-	-	3000	80	40	-	-	-	<1	50	6	>2420	80	33	<1	54	9
1290	3 2 1	-	-	-	1000	7	19	-	-	-	<1	7	7	-	-	-	-	-	-
1545	1 2 3	2700	79	33	2700	79	33	730	37	18	0	33	11	3150	91	30	0	43	11
1594	2 3 1	2400	61	23	2400	61	23	260	29	23	<1	39	8	2200	74	32	<1	45	9
1611	3 1 2	4200	80	35	4200	80	35	950	45	28	0	34	10	2908	81	32	0	37	11
1753	2 1 3	2000	78	36	2000	78	36	-	-	-	0	36	18	3870	91	45	0	44	16
1868	3 2 1	3900	97	26	3900	97	26	-	-	-	0	55	11	4635	62	35	0	35	8
1970	2 3 1	3500	84	20	3500	84	20	1300	43	15	0	0	11	-	-	-	-	-	-
2050	3 1 2	-	-	-	2645	89	31	-	-	-	0	54	11	2851	70	28	0	50	9
2386	1 2 3	1800	86	28	1800	86	28	-	-	-	0	50	10	-	-	-	-	-	-
2637	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	5600	66	22	<1,0	43	5
2704	2 1 3	-	-	-	1660	41	26	-	-	-	0	41	8	2005	70	24	<1	41	10
2745	3 2 1	580	63	21	580	63	21	0	35	21	0	35	7	-	-	-	-	-	-
2797	3 1 2	1591	66	23	1591	66	23	0	34	7	0	34	7	2909	55	38	0	36	10
2915	3 1 2	-	-	-	620	2	10	-	-	-	<1	<1	<1	-	-	-	-	-	-
2944	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	1652	78.2	34.4	<1	45.3	17.8
3055	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	2420	50	4	0	38	0
3159	1 3 2	-	-	-	2000	60	32	-	-	-	0	35	10	-	-	-	-	-	-
3162	1 3 2	1900	88	35	1900	88	35	-	-	-	0	56	19	3690	46	17	0	36	3
3305	3 1 2	-	-	-	2800	85	32	-	-	-	<1	48	14	3600	59	18	<1	48	5
3339	2 3 1	1600	78	25	1600	78	25	-	-	-	0	34	11	-	-	-	-	-	-
3730	2 3 1	1200	53	26	-	-	-	900	37	18	-	-	-	-	-	-	-	-	-
3868	3 1 2	2700	67	19	2700	67	19	700	43	18	0	37	9	3600	42	31	0	27	18
4015	2 3 1	3450	62	17	3450	62	17	-	-	-	1753	12	6	3200	61	25	0	39	12
4288	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	3 1 2	3600	73	35	3600	73	35	1230	41	25	0	39	18	3800	70	28	0	40	13
4339	1 2 3	3700	80	30	3700	80	30	<100	37	28	0	32	15	3450	82	26	0	50	6
4343	1 2 3	2500	74	32	2500	74	32	-	-	-	0	45	10	4106	77	31	0	50	12
4356	3 1 2	2800	96	40	2800	96	40	340	41	24	0	0	19	3300	83	25	<1	46	15
4723	3 2 1	1545	77	22	1545	77	22	-	-	-	0	38	10	4352	93	39	0	47	11
4889	3 2 1	-	-	-	1800	70	21	-	-	-	0	33	12	2400	82	42	0	43	23
4980	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	1920	73.8	20.3	<1	50.4	4.2
5018	2 3 1	3400	86	36	680	0	36	-	-	-	0	0	0	3466	102	70	0	83	17
5120	2 3 1	-	-	-	2800	64	33	1300	33	20	0	34	7	3300	75	29	0	41	7
5128	3 2 1	-	-	-	-	-	-	1200	25	26	<1	25	26	3060	41	32	<1	29	14
5201	2 3 1	140	38	13	0	38	13	-	-	-	0	14	9	-	-	-	-	-	-
5352	2 3 1	-	-	-	1850	1832	71	-	-	-	0	40	13	-	-	-	-	-	-
5447	1 3 2	-	-	-	3000	200	30	-	-	-	0	200	16	-	-	-	-	-	-
5553	3 2 1	-	-	-	-	-	15	-	-	-	-	-	2	-	-	-	-	-	-
5858	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	2900	70	30	<1	39	16
5950	3 1 2	3200	90	25	3200	90	25	427	25	24	0	49	11	-	-	-	-	-	-
6175	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	200	66	29	0	50	11
6180	3 1 2	3800	80	25	3800	80	25	1700	35	29	0	40	13	3600	95	31	0	50	10
6233	3 1 2	3000	89	40	3000	89	40	-	-	-	0	48	19	2100	70	35	0	38	12
6253	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	2300	71	29	0	64	7
6448	3 2 1	2850	110	30	-	-	-	-	-	-	0	20	14	-	-	-	-	-	-
6456	2 3 1	-	-	-	850	90	27	-	-	-	0	8	11	3400	118	32	0	70	16
6563	1 2 3	2040	60	39	2040	60	39	2040	60	39	<1	48	23	4645	112	40	<1	65	24
6686	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	>200.5	38.4	19.2	<1	32.4	4.2
7248	2 3 1	2027	61	34	1400	57	34	770	27	30	<1	26	22	3270	93	29.8	<1	49	13.4
7302	3 1 2	3450	84	31	3450	84	31	<1	51	31	<1	39	13	2900	77	30	<1	38	10
7442	3 2 1	3500	71	23	3500	71	23	-	-	-	0	42	12	2424	75	35	0	52	14
7688	3 2 1	-	-	-	1500	62	16	-	-	-	0	41	8	2600	46	27	0	33	10
7728	1 2 3	-	-	-	1500	44	25	-	-	-	0	22	6	-	-	-	-	-	-
7876	3 1 2	3000	77	38	3000	77	38	1136	25	27	<1	36	17	4350	67	34	<1	36	22
7896	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7930	1 3 2	2070	65	25	2070	65	25	-	-	-	<1	38	13	>2000	83	48	<1	56	27
7962	3 1 2	2100	87	37	2100	87	37	360	27	23	0	41	8	>2420	78	33	0	40	10
7968	1 2 3	2754	52	38	2754	52	38	234	24	21	0	24	8	2630	54	45	0	28	9
8068	3 2 1	-	-	-	2400	65	37	790	31	30	0	37	37	3400	63	39	0	44	23
8252	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	2900	74	19	<1	36	9
8260	3 2 1	1345	67	26	1345	67	26	-	-	-	<1,0	37	3	-	-	-	-	-	-
8329	2 1 3	2736	90	28	2736	90	28	-	-	-	0	55	13	2608	84	37	0	56	10
8380	2 4 3	-	67	-	-	67	-	-	-	-	-	28	-	-	78	-	-	38	-
8435	2 1 3	-	-	-	3000	65	36	220	21	19	0	35	12	-	-	-	-	-	-
Mean					2242	70	28				0	36	12	3170	73	31	0	44	12
CV (%)					23	12	15				-	18	24	12	12	12	-	11	22

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 of a laboratory are obtained as the square roots of each 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.

Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22±2 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
0	98	440	-	-	-	-	-	-	-	-	-	-	-	-	98	12	0	1131
<1	1	30	<1	<1	30	-	-	-	-	-	-	-	-	-	110	18	<1	1237
-	-	-	<1	<1	420	-	-	-	-	-	-	-	-	-	32	30	1	1290
0	20	800	0	0	800	540	0	65	1500	0	0	0	55	0	71	28	4	1545
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	22	3	1594
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	21	0	1611
0	124	460	-	-	-	1000	0	60	1100	0	0	0	55	0	96	22	1	1753
0	528	471	-	-	-	-	0	68	657	0	0	0	67	0	101	20	3	1868
0	610	230	0	0	230	900	0	40	900	0	0	-	-	-	120	17	1	1970
0	2280	436	-	-	-	10	0	62	1073	0	0	0	72	0	124	30	1	2050
0	80	61	0	0	61	-	-	-	-	-	-	-	-	-	121	31	2	2386
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	140	28	14	2637
-	-	-	<1	<1	90	-	-	-	-	-	-	-	-	-	48	23	1	2704
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	21	0	2745
705	3800	480	0	0	480	0	0	85	1064	0	0	-	-	-	66	20	2	2797
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31	40	6	2915
-	-	-	<1	<1	91	-	-	-	-	-	-	-	-	-	38	22	3	2944
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	87	21	2	3055
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3076
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3145
-	-	-	0	0	88	-	-	-	-	-	-	-	-	-	112	26	2	3159
0	280	440	-	-	-	410	2	60	800	0	0	0	61	0	90	23	3	3162
-	-	-	<1	<1	300	790	<1	150	<1	<1	<1	<1	76	<1	140	27	1	3305
0	21	540	0	0	540	-	-	-	-	-	-	-	-	-	100	27	0	3339
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	82	16	0	3730
0	67	430	0	0	430	0	0	130	900	0	0	0	55	0	80	15	1	3868
0	1750	463	-	-	-	333	0	77	793	0	0	0	39	0	91	21	2	4015
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	15	<1	4288
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	83	23	1	4319
0	0	250	0	0	250	900	0	89	850	0	0	-	-	-	105	25	3	4339
0	210	373	-	-	-	378	0	56	802	0	0	0	54	0	85	21	0	4343
0	1500	320	0	0	320	-	-	-	-	-	-	-	-	-	68	19	1	4356
0	1000	236	-	-	-	545	0	40	636	0	0	0	82	0	99	27	0	4723
-	-	-	0	0	53	-	-	-	-	-	-	-	-	-	110	19	3	4889
0	520	52	<1	<1	52	-	-	-	-	-	-	-	-	-	84	22	2	4980
0	700	440	0	0	440	270	0	115	765	0	0	0	82	0	187	21	2	5018
-	-	-	0	1400	360	550	0	62	870	0	0	0	92	0	100	20	0	5120
-	-	-	<1	<1	110	-	-	-	-	-	-	-	-	-	97	26	3	5128
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	22	0	5201
0	1870	440	-	-	-	510	0	150	840	0	0	0	90	0	65	22	1	5352
0	2900	600	0	0	600	180	0	55	600	0	0	0	44	0	83	16	3	5447
-	-	-	-	-	400	-	-	-	-	-	-	-	-	-	-	-	-	5553
<1	<1	57	<1	<1	57	-	-	-	-	-	-	-	-	-	41	21	3	5858
0	1027	245	0	0	245	700	0	49	764	0	0	0	71	0	89	19	0	5950
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	195	37	2	6175
0	0	210	0	0	210	-	-	-	-	-	-	-	-	-	100	21	1	6180
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95	15	0	6233
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	21	1	6253
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	32	2	6448
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	112	18	1	6456
<1	1400	377	-	-	-	580	<1	140	800	<1	<1	<1	73	<1	86	32	1	6563
<1	640	310	-	-	-	-	-	-	-	-	-	-	-	-	160	25	2	6686
<1	2100	318	-	-	-	<1	<1	55	1582	<1	<1	<1	64	<1	65	24	<1	7248
<1	2200	527	-	-	-	282	<1	46	854	<1	<1	<1	64	<1	72	14	1	7302
0	200	455	-	-	-	-	-	-	-	-	-	0	63	0	105	19	0	7442
-	-	-	0	0	170	370	0	41	750	0	0	0	130	0	90	20	1	7688
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60	20	3	7728
<1	2600	282	-	-	-	400	<1	100	850	<1	<1	<1	70	<1	67	24	2	7876
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7896
<1	2000	340	<1	<1	<1	600	<1	210	960	<1	<1	-	-	-	110	18	1	7930
-	-	-	-	-	-	0	0	85	1140	0	0	-	-	-	49	28	1	7962
0	1905	275	0	0	275	-	-	-	-	-	-	-	-	-	69	20	2	7968
-	-	-	0	0	530	0	0	80	900	0	0	-	-	-	78	20	1	8068
-	-	-	<1	<1	33	-	-	-	-	-	-	-	-	-	100	20	3	8252
-	-	-	<1	<1	275	-	-	-	-	-	-	-	-	-	49	21	2	8260
0	118	348	-	-	-	650	0	39	950	0	0	0	52	0	79	26	0	8329
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24	-	-	8380
-	-	-	0	0	170	-	-	-	-	-	-	-	-	-	73	26	1	8435
0	790	314	0	0	232	495	0	73	866	0	0	0	68	0	85	22	1	Mean
-	69	27	-	-	40	21	-	23	8	-	-	-	13	-	19	12	70	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
8569	3 2 1	3500	62	34	3500	62	34	244	18	18	0	18	8	-	-	-	-	-	-
8626	2 3 1	500	37	30	450	33	15	0	0	15	0	0	15	-	-	-	-	-	-
8628	3 2 1	-	-	-	2000	79	35	-	-	-	0	48	15	-	-	-	-	-	-
8663	2 3 1	2300	81	35	2300	81	35	2100	58	32	0	41	18	4100	57	31	0	36	13
8742	1 2 3	-	-	-	1900	46	24	-	-	-	0	30	6	-	-	-	-	-	-
8751	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	2755	83	31	<1	43	11
8766	3 1 2	3000	75	32	3000	75	32	1300	25	22	<1	40	13	3870	88	58	<1	43	21
8840	1 3 2	3400	85	43	3400	85	43	-	-	-	0	43	22	3800	70	38	0	45	15
8862	3 1 2	3636	77	31	3636	77	31	-	-	-	0	37	17	2644	97	39	0	56	9
8898	2 1 3	2909	79	24	2909	79	24	-	-	-	0	49	8	3610	95	25	0	46	8
8955	2 3 1	-	-	-	1700	40	18	610	30	25	0	40	18	3600	68	32	0	50	16
8998	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	>2419,6	75,9	22,6	<1	36,2	4,65
9051	3 2 1	-	-	-	1700	58	18	-	-	-	0	27	5	-	-	-	-	-	-
9436	3 1 2	256	63	18	256	63	18	<1	25	22	<1	41	8	488	68	39	<1	46	10
9524	2 1 3	-	-	-	1460	62	28	-	-	-	<1	39	6	2650	57	28	<1	43	10
9736	3 2 1	2909	44	18	2909	44	18	-	-	-	0	18	5	2946	92	27	0	30	11
9899	2 3 1	2658	107	30	2658	107	30	-	-	-	0	67	17	2916	64	44	0	45	13
9903	1 3 2	2792	73	36	2792	73	36	62	33	33	0	33	15	-	-	-	-	-	-

n	47	48	47	66	67	67	30	30	30	68	69	69	53	59	58	58	59	58
Min	140	37	13	0	0	10	0	0	7	0	0	0	200	38,4	4	0	27	0
Max	4200	110	43	4200	1832	71	2100	60	39	1753	200	37	5600	118	70	0	83	27
Median	2736	77	30	2500	73	29	655	33	23,5	0	37	11	3200	74	31	0	43	11
Mean				2242	70	28				0	36	12	3170	73	31	0	44	12
CV (%)				23	12	15				-	18	24	12	12	12	-	11	22
False positive				0	0	0				1	0	0	0	0	0	0	0	0
False negative				1	1	0				0	5	2	0	0	0	0	0	1
Outliers, low				0	2	0				0	0	0	2	0	1	0	0	0
Outliers, high				0	2	1				0	1	0	0	0	2	0	0	0
Low limit OK	140	37	13	256	33	10	0	0	7	0	7	2	1652	38	17	0	27	3
High limit OK	4200	110	43	4200	107	43	2100	60	39	0	67	37	5600	118	48	0	83	27

mv ($\sqrt{\text{Mean}}$)				47.346	8.381	5.255				0.000	5.964	3.418	56.305	8.534	5.553	0.000	6.623	3.392
s ($\text{CV} \cdot \text{mv} / 100$)				10.775	0.968	0.772				0.000	1.070	0.819	6.830	0.983	0.646	0.000	0.730	0.751
u _{rel,mv} (%) ($100 \cdot s / \sqrt{n_{mv}}$)				2.8	1.5	1.8				2.3	2.9		1.7	1.5	1.6		1.4	2.9
x ($\sqrt{\text{Result}}$)																		
z ($[(x-mv)/s]$)																		

Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22±2 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
0	1010	350	-	-	-	-	-	-	-	-	-	-	-	113	31	58	8569	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	38	18	2	8626	
-	-	-	0	0	47	800	0	40	770	0	0	-	-	116	33	2	8628	
0	1500	250	0	0	250	-	-	-	-	-	-	-	-	83	26	5	8663	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	34	2	8742	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	121	24	<1	8751	
<1	2100	455	-	-	-	336	<1	118	880	<1	<1	<1	106	<1	66	19	3	8766
-	-	-	-	-	-	-	-	-	-	-	-	-	-	138	24	1	8840	
0	1700	355	0	0	355	255	0	60	874	0	0	0	67	0	114	23	4	8862
0	1396	330	-	-	-	555	0	81	1064	0	0	0	66	0	113	21	2	8898
-	-	-	0	0	460	340	0	57	1000	0	0	0	49	0	74	13	1	8955
<1	<1	142	-	-	-	-	-	-	-	-	-	-	-	49	22	1	8998	
-	-	-	0	0	360	-	-	-	-	-	-	-	-	65	22	1	9051	
<1	2600	227	-	-	-	445	<1	41	700	<1	<1	<1	69	40	67	23	<1	9436
<1	5600	74	<1	<1	74	-	-	-	-	-	-	-	-	100	26	3	9524	
0	0	306	-	-	-	600	0	64	1082	0	0	0	74	0	77	15	1	9736
0	1118	333	-	-	-	347	0	53	745	0	0	0	64	0	74	21	0	9899
0	1280	246	-	-	-	400	0	58	1020	0	0	0	62	0	131	21	1	9903
44	44	44	36	36	37	35	36	36	36	36	36	30	30	30	81	82	81	n
0	0	30	0	0	0	0	0	39	0	0	0	0	39	0	31	12	0	Min
705	5600	800	0	1400	800	1000	2	210	1582	0	0	0	130	40	195	40	58	Max
0	1005	336.5	0	0	250	510	0	62	854	0	0	0	66.5	0	86	22	1	Median
0	790	314	0	0	232	495	0	73	866	0	0	0	68	0	85	22	1	Mean
-	69	27	-	-	40	21	-	23	8	-	-	-	13	-	19	12	70	CV (%)
1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	False pos.
0	0	0	0	0	1	5	0	0	1	0	0	0	0	0	0	0	0	False neg.
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	Outliers <
0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	1	Outliers >
0	0	30	0	0	30	180	0	39	600	0	0	0	39	0	31	12	0	Low limit
0	5600	800	0	0	800	1000	0	210	1140	0	0	0	130	0	195	40	14	High limit
0.000	28.111	17.712	0.000	0.000	15.224	22.258	0.000	8.563	29.424	0.000	0.000	0.000	8.238	0.000	9.205	4.731	1.050	mv
0.000	19.340	4.824	0.000	0.000	6.111	4.623	0.000	2.010	2.383	0.000	0.000	0.000	1.052	0.000	1.735	0.547	0.736	s
	10.4	4.1			6.7	3.9		3.9	1.4				2.3		2.1	1.3	7.8	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				0.338	-0.015	0.747							0.000	0.111	0.861	0.607	-0.110	-1.171	0.000	0.116	0.097
1237				0.689	0.582	1.387							0.000	1.034	-1.182		0.417	0.297	0.000	0.993	-0.522
1290				-1.459	-4.000	-1.161							0.000	-3.100	-0.943						
1545				0.428	0.524	0.635							0.000	-0.205	-0.124	-0.026	1.022	-0.117	0.000	-0.090	-0.100
1594				0.153	-0.589	-0.594							0.000	0.262	-0.720	-1.377	0.069	0.161	0.000	0.116	-0.522
1611				1.621	0.582	0.857							0.000	-0.124	-0.312	-0.348	0.474	0.161	0.000	-0.740	-0.100
1753				-0.244	0.466	0.966							0.000	0.034	1.007	0.865	1.022	1.788	0.000	0.013	0.810
1868				1.402	1.516	-0.202							0.000	1.357	-0.124	1.724	-0.672	0.562	0.000	-0.969	-0.750
1970				1.097	0.810	-1.014							0.000		-0.124						
2050				0.379	1.088	0.406							0.000	1.293	-0.124	-0.426	-0.171	-0.404	0.000	0.613	-0.522
2386				-0.457	0.922	0.048							0.000	1.034	-0.312						
2637																2.713	-0.417	-1.334	0.000	-0.090	-1.539
2704				-0.613	-2.042	-0.202							0.000	0.410	-0.720	-1.688	-0.171	-1.011	0.000	-0.302	-0.306
2745				-2.159	-0.458	-0.871							0.000	-0.045	-0.943						
2797				-0.692	-0.265	-0.594							0.000	-0.124	-0.943	-0.347	-1.137	0.947	0.000	-0.854	-0.306
2915				-2.083	-4.000	-2.711							0.000								
2944																-2.293	0.314	0.484	0.000	0.147	1.102
3055																					
3076																					
3145																-1.041	-1.488	-4.000	0.000	-0.629	
3159				-0.244	-0.656	0.521							0.000	-0.045	-0.312						
3162				-0.349	1.033	0.857							0.000	1.419	1.149	0.650	-1.782	-2.212	0.000	-0.854	-2.211
3305				0.517	0.866	0.521							0.000	0.901	0.395	0.541	-0.868	-2.027	0.000	0.417	-1.539
3339				-0.682	0.466	-0.330							0.000	-0.124	-0.124						
3730																					
3868				0.428	-0.202	-1.161							0.000	0.111	-0.510	0.541	-2.088	0.023	0.000	-1.954	1.134
4015				1.057	-0.523	-1.466							-2.336	-1.182		0.039	-0.736	-0.855	0.000	-0.518	0.097
4288																					
4319				1.174	0.169	0.857							0.000	0.262	1.007	0.782	-0.171	-0.404	0.000	-0.409	0.285
4339				1.251	0.582	0.289							0.000	-0.287	0.556	0.356	0.530	-0.702	0.000	0.613	-1.255
4343				0.246	0.229	0.521							0.000	0.695	-0.312	1.138	0.245	0.023	0.000	0.613	0.097
4356				0.517	1.463	1.387							0.000		1.149	0.167	0.586	-0.855	0.000	0.218	0.641
4723				-0.746	0.407	-0.731							0.000	0.187	-0.312	1.415	1.128	1.071	0.000	0.318	-0.100
4889				-0.457	-0.015	-0.871							0.000	-0.205	0.056	-1.071	0.530	1.436	0.000	-0.090	1.871
4980																-1.828	0.057	-1.620	0.000	0.651	-1.788
5018				-1.974		0.966							0.000			0.376	1.592	4.000	0.000	3.405	0.974
5120				0.517	-0.393	0.635							0.000	-0.124	-0.943	0.167	0.128	-0.259	0.000	-0.302	-0.994
5128													0.000	-0.901	2.052	-0.145	-2.167	0.161	0.000	-1.695	0.466
5201					-2.289	-2.137							0.000	-2.076	-0.510						
5352				-0.402	4.000	4.000							0.000	0.337	0.229						
5447				0.689	4.000	0.289							0.000	4.000	0.711						
5553						-1.790									-2.446						
5858																-0.359	-0.171	-0.117	0.000	-0.518	0.810
5950				0.856	1.142	-0.330							0.000	0.968	-0.124						
6175																-4.000	-0.417	-0.259	0.000	0.613	-0.100
6180				1.327	0.582	-0.330							0.000	0.337	0.229	0.541	1.233	0.023	0.000	0.613	-0.306
6233				0.689	1.088	1.387							0.000	0.901	1.149	-1.534	-0.171	0.562	0.000	-0.629	0.097
6253																-1.222	-0.110	-0.259	0.000	1.885	-0.994
6448													0.000	-1.394	0.395						
6456				-1.688	1.142	-0.076							0.000	-2.930	-0.124	0.294	2.368	0.161	0.000	2.386	0.810
6563				-0.202	-0.656	1.283							0.000	0.901	1.682	1.735	2.083	1.194	0.000	1.970	2.008
6686																-2.377	-1.812		0.000	-1.275	-1.788
7248				-0.922	-0.858	0.747							0.000	-0.808	1.554	0.129	1.128	-0.145	0.000	0.516	0.358
7302				1.057	0.810	0.406							0.000	0.262	0.229	-0.359	0.245	-0.117	0.000	-0.629	-0.306
7442				1.097	0.047	-0.594							0.000	0.483	0.056	-1.035	0.128	0.562	0.000	0.805	0.466
7688				-0.800	-0.523	-1.626							0.000	0.410	-0.720	-0.778	-1.782	-0.552	0.000	-1.203	-0.306
7728				-0.800	-1.805	-0.330							0.000	-1.190	-1.182						
7876				0.689	0.407	1.179							0.000	0.034	0.861	1.413	-0.355	0.431	0.000	-0.854	1.730
7896																					
7930				-0.172	-0.329	-0.330							0.000	0.187	0.229		0.586	2.128	0.000	1.177	2.404
7962				-0.141	0.977	1.073							0.000	0.410	-0.720	0.303	0.297		0.000	-0.409	-0.306
7968				0.476	-1.208	1.179							0.000	-0.995	-0.720	-0.735	-1.206	1.788	0.000	-1.824	-0.522
8068				0.153	-0.329	1.073							0.000	0.111	3.254	0.294	-0.607	1.071	0.000	0.013	1.871
8252																-0.359	0.069	-1.847	0.000	-0.854	-0.522
8260				-0.990	-0.202	-0.202							0.000	0.111	-2.058						
8329				0.460	1.142	0.048							0.000	1.357	0.229	-0.767	0.642	0.820	0.000	1.177	-0.306
8380					-0.202									-0.628			0.303				-0.629
8435				0.689	-0.329	0.966							0.000	-0.045	0.056						
8569				1.097	-0.523	0.747							0.000	-1.608	-0.720						
8626				-2.425	-2.722	-1.790							0.000		0.556						
8628				-0.244	0.524	0.857							0.000	0.901	0.556						
8663				0.057	0.639	0.857							0.000	0.410	1.007	1.131	-1.001	0.023	0.000	-0.854	0.285
8742				-0.349	-1.651	-0.461							0.000	-0.455	-1.182						
8751																-0.559	0.586	0.023	0.000	-0.090	-0.100
8766				0.689	0.289	0.521							0.000	0.337	0.229	0.865	0.861	3.192	0.000	-0.090	1.587
8840				1.018	0.866	1.688							0.000	0.554	1.554	0.782	-0.171	0.947	0.000	0.116	0.641
8862				1.202	0.407	0.406							0.000	0.111	0.861	-0.715	1.337	1.071	0.000	1.177	-0.522
8898				0.612	0.524	-0.461							0.000	0.968	-0.720	0.553	1.233	-0.855	0.000	0.218	-0.750
8955				-0.568	-2.123	-1.311							0.000	0.337	1.007	0.541	-0.293	0.161	0.000	0.613	0.810
8998																					

positive results no z-scores can 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.

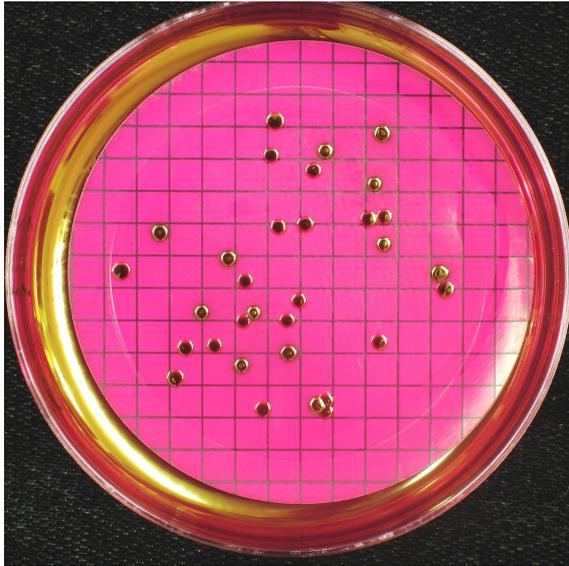
Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0.000	-0.942	0.677	0.000	0.000	-1.595										0.400	-2.316	-1.426	1131
0.000	-1.402	-2.537	0.000	0.000	0.862										0.740	-0.893	-1.426	1237
			0.000	0.000											-2.045	1.363	-0.068	1290
0.000	-1.222	2.192	0.000	0.000	2.137	0.212	0.000	-0.249	3.905	0.000	0.000	0.000	-0.781	0.000	-0.449	1.023	1.291	1545
															0.458	-0.075	0.927	1594
															-1.230	-0.272	-1.426	1611
0.000	-0.878	0.774				2.026	0.000	-0.406	1.570	0.000	0.000	0.000	-0.781	0.000	0.342	-0.075	-0.068	1753
0.000	-0.265	0.827					0.000	-0.158	-1.591	0.000	0.000	0.000	-0.050	0.000	0.487	-0.474	0.927	1868
0.000	-0.177	-0.528	0.000	0.000	-0.010	1.675	0.000	-1.114	0.242	0.000	0.000	0.000			1.008	-1.112	-0.068	1970
0.000	1.015	0.657				-4.000	0.000	-0.343	1.398	0.000	0.000	0.000	0.235	0.000	1.113	1.363	-0.068	2050
0.000	-0.991	-2.053	0.000	0.000	-1.213										1.035	1.528	0.495	2386
															1.514	1.023	3.656	2637
			0.000	0.000	-0.939										-1.312	0.118	-0.068	2704
															-1.570	-0.272	-1.426	2745
	1.734	0.870	0.000	0.000	1.094		0.000	0.327	1.340	0.000	0.000	0.000			-0.623	-0.474	0.495	2797
			0.000	0.000	-0.930										-2.096	2.911	1.901	2915
															-1.753	-0.075	0.927	2944
															0.071	-0.272	0.495	3055
																		3076
																		3145
			0.000	0.000	-0.956										0.794	0.672	0.495	3159
0.000	-0.588	0.677				-0.435		-0.406	-0.478	0.000	0.000	0.000	-0.407	0.000	0.163	0.118	0.927	3162
			0.000	0.000	0.343	1.265	0.000	1.833		0.000	0.000	0.000	0.456	0.000	1.514	0.849	-0.068	3305
0.000	-1.217	1.146	0.000	0.000	1.311										0.458	0.849	-1.426	3339
															-0.086	-1.337	-1.426	3730
0.000	-1.030	0.627	0.000	0.000	0.902		0.000	1.412	0.242	0.000	0.000	0.000	-0.781	0.000	-0.150	-1.569	-0.068	3868
0.000	0.710	0.789				-0.867	0.000	0.106	-0.530	0.000	0.000	0.000	-1.895	0.000	0.193	-0.272	0.495	4015
															-0.659	-1.569	-1.426	4288
															-0.055	0.118	-0.068	4319
0.000	-1.454	-0.394	0.000	0.000	0.096	1.675	0.000	0.433	-0.113	0.000	0.000	0.000			0.601	0.491	0.927	4339
0.000	-0.704	0.332				-0.609	0.000	-0.537	-0.464	0.000	0.000	0.000	-0.846	0.000	0.008	-0.272	-1.426	4343
0.000	0.549	0.037	0.000	0.000	0.436										-0.553	-0.681	-0.068	4356
0.000	0.182	-0.487				0.235	0.000	-1.114	-1.765	0.000	0.000	0.000	0.777	0.000	0.429	0.849	-1.426	4723
			0.000	0.000	-1.300										0.740	-0.681	0.927	4889
0.000	-0.274	-2.177	0.000	0.000	-1.311										-0.023	-0.075	0.495	4980
0.000	-0.086	0.677	0.000	0.000	0.941	-1.260	0.000	1.075	-0.741	0.000	0.000	0.000	0.777	0.000	2.576	-0.272	0.495	5018
			0.000	0.000	0.614	0.258	0.000	-0.343	0.030	0.000	0.000	0.000	1.287	0.000	0.458	-0.474	-1.426	5120
			0.000	0.000	-0.775										0.371	0.672	0.927	5128
															-1.312	-0.075	-1.426	5201
0.000	0.783	0.677	0.000	0.000	1.517	0.070	0.000	1.833	-0.185	0.000	0.000	0.000	1.187	0.000	-0.659	-0.075	-0.068	5352
0.000	1.331	1.406			0.782	-1.913	0.000	-0.571	-2.068	0.000	0.000	0.000	-1.525	0.000	-0.055	-1.337	0.927	5447
																		5553
0.000	-1.454	-2.107	0.000	0.000	-1.256										-1.615	-0.272	0.927	5858
0.000	0.204	-0.427	0.000	0.000	0.070	0.908	0.000	-0.778	-0.749	0.000	0.000	0.000	0.179	0.000	0.132	-0.681	-1.426	5950
															2.743	2.469	0.495	6175
0.000	-1.454	-0.668	0.000	0.000	-0.120										0.458	-0.272	-0.068	6180
															0.312	-1.569	-1.426	6233
															0.163	-0.272	-0.068	6253
															-1.706	1.691	0.495	6448
															0.794	-0.893	-0.068	6456
0.000	0.481	0.353				0.395	0.000	1.627	-0.478	0.000	0.000	0.000	0.291	0.000	0.040	1.691	-0.068	6563
0.000	-0.145	-0.022													1.985	0.491	0.495	6686
0.000	0.916	0.025							4.000	0.000	0.000	0.000	-0.226	0.000	-0.659	0.306	-1.426	7248
0.000	0.972	1.087				-1.182	0.000	-0.886	-0.084	0.000	0.000	0.000	-0.226	0.000	-0.415	-1.809	-0.068	7302
0.000	-0.722	0.750													0.601	-0.681	-1.426	7442
			0.000	0.000	-0.358	-0.654	0.000	-1.075	-0.855	0.000	0.000	0.000	3.008	0.000	0.163	-0.474	-0.068	7688
															-0.841	-0.474	0.927	7728
0.000	1.183	-0.191				-0.489	0.000	0.715	-0.113	0.000	0.000	0.000	0.122	0.000	-0.588	0.306	0.495	7876
																		7896
0.000	0.859	0.151	0.000	0.000		0.484	0.000	2.950	0.654	0.000	0.000	0.000			0.740	-0.893	-0.068	7930
							0.000	0.327	1.821	0.000	0.000	0.000			-1.271	1.023	-0.068	7962
0.000	0.803	-0.234	0.000	0.000	0.222										-0.518	-0.474	0.495	7968
			0.000	0.000	1.276		0.000	0.190	0.242	0.000	0.000	0.000			-0.215	-0.474	-0.068	8068
			0.000	0.000	-1.551										0.458	-0.474	0.927	8252
			0.000	0.000	0.222										-1.271	-0.272	0.495	8260
0.000	-0.892	0.195				0.700	0.000	-1.153	0.586	0.000	0.000	0.000	-0.976	0.000	-0.183	0.672	-1.426	8329
															0.306			8380
			0.000	0.000	-0.358										-0.381	0.672	-0.068	8435
0.000	0.190	0.207													0.822	1.528	4.000	8569
															-1.753	-0.893	0.495	8626
0.000	0.549	-0.394	0.000	0.000	-1.369	1.304	0.000	-1.114	-0.703	0.000	0.000	0.000			0.902	1.851	0.495	8628
			0.000	0.000	0.096										-0.055	0.672	1.611	8663
															-1.526	2.009	0.495	8742
0.000	0.916	0.750				-0.850	0.000	1.144	0.101	0.000	0.000	0.000	1.956	0.000	1.035	0.306	-1.426	8751
															-0.623	-0.681	0.927	8766
															1.465	0.306	-0.068	8840
0.000	0.678	0.234	0.000	0.000	0.592	-1.361	0.000	-0.406	0.058	0.000	0.000	0.000	-0.050	0.000	0.849	0.118	1.291	8862
0.000	0.478	0.094				0.281	0.000	0.218	1.340	0.000	0.000	0.000	-0.108	0.000	0.822	-0.272	0.495	8898
			0.000	0.000	1.018	-0.826	0.000	-0.504	0.922	0.000	0.000	0.000	-1.177	0.000	-0.347	-2.057	-0.068	8955
0.000	-1.454	-1.202													-1.271	-0.075	-0.068	8998
			0.000	0.000	0.614										-0.659	-0.075	-0.068	9051
0.000	1.183	-0.549	0.000	0.000	-1.084	-0.252	0.000	-1.075	-1.245	0.000	0.000	0.000	0.065	4.000	-0.588	0.118	-1.426	9436
0.000	2.416	-1.889													0.458	0.672	0.927	9524
0.000	-1.454	-0.045				0.484	0.000	-0.280	1.456	0.000	0.000	0.000	0.346	0.000	-0.248	-1.569	-0.068	9736
0.000	0.275	0.111				-0.785	0.000	-0.638	-0.894	0.000	0.000	0.000	-0.226	0.000	-0.347	-0.272	-1.426	9899

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
9903					0.510	0.169	0.966				0.000	-0.205	0.556						
n		0	0	0	65	66	67	0	0	0	67	64	67	53	59	58	58	59	57
Min					-2.909	-4.000	-2.711				0.000	-3.100	-2.446	-4.000	-2.377	-4.000	0.000	-1.954	-2.211
Max					1.621	4.000	4.000				0.000	4.000	3.254	2.713	2.368	4.000	0.000	3.405	2.404
Median					0.246	0.169	0.289				0.000	0.149	-0.124	-0.026	0.069	0.023	0.000	-0.090	-0.100
Mean					0.000	0.000	0.060				0.000	0.063	0.000	-0.151	0.000	0.055	0.000	0.000	0.000
SD					1.000	1.387	1.106				0.000	1.111	1.000	1.246	1.000	1.298	0.000	1.000	1.000
z<-3					0	2	0				0	1	0	2	0	1	0	0	0
-3≤z<-2					4	4	2				0	3	2	1	3	2	0	0	1
-2≤z≤3					0	1	0				0	1	1	1	2	1	0	1	2
z>3					0	2	1				0	1	1	0	0	2	0	1	0

Presumptive C. <i>perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0.000	0.396	-0.420				-0.489	0.000	-0.471	1.054	0.000	0.000	0.000	-0.346	0.000	1.291	-0.272	-0.068	9903
43	44	44	36	35	36	30	35	36	35	36	36	30	30	30	81	82	81	n
0.000	-1.454	-2.537	0.000	0.000	-1.595	-4.000	0.000	-1.153	-2.068	0.000	0.000	0.000	-1.895	0.000	-2.096	-2.316	-1.426	Min
0.000	2.416	2.192	0.000	0.000	2.137	2.026	0.000	2.950	4.000	0.000	0.000	0.000	3.008	4.000	2.743	2.911	4.000	Max
0.000	0.186	0.131	0.000	0.000	0.096	-0.091	0.000	-0.343	0.030	0.000	0.000	0.000	-0.079	0.000	0.040	-0.075	-0.068	Median
0.000	0.000	0.000	0.000	0.000	0.001	-0.133	0.000	0.000	0.226	0.000	0.000	0.000	0.000	0.133	0.000	0.000	0.066	Mean
0.000	1.000	1.000	0.000	0.000	1.000	1.224	0.000	1.000	1.344	0.000	0.000	0.000	1.000	0.730	1.000	1.000	1.076	SD
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	7
0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	31
0	1	1	0	0	1	1	0	1	0	0	0	0	0	0	2	3	0	20
0	0	0	0	0	0	0	0	0	2	0	0	0	1	1	0	0	2	14

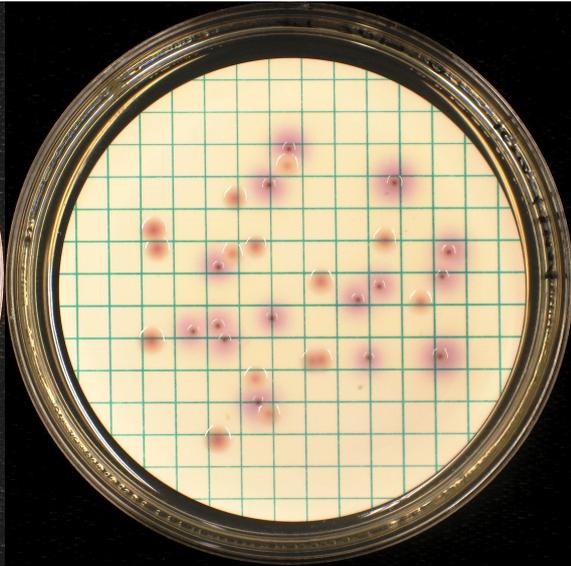
Mixture A

m-Endo Agar LES, 37 °C



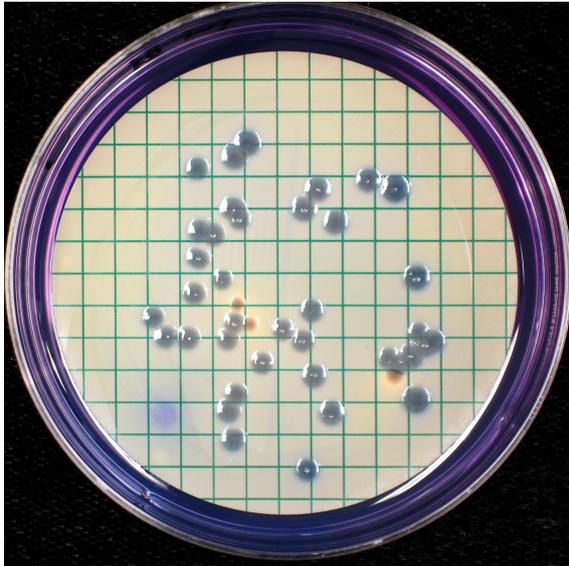
1 ml

Chromocult Coliform Agar, 37 °C



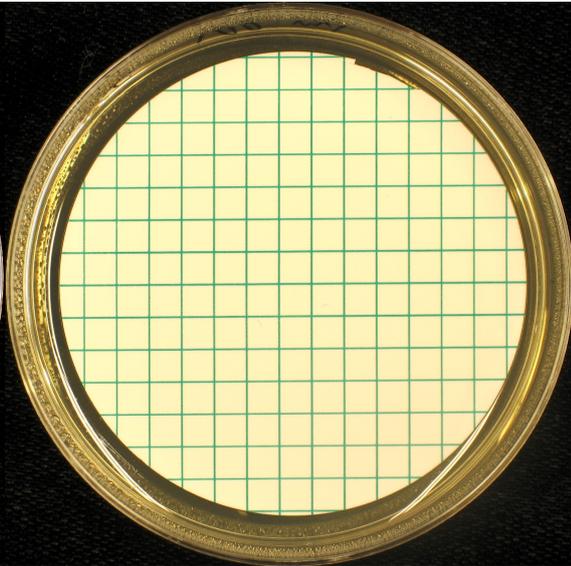
1 ml

m-FC Agar, 44 °C



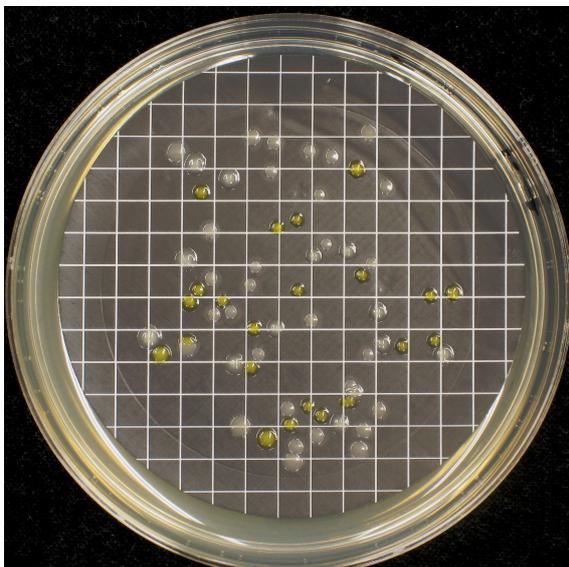
10 ml

m-TSC Agar, 44 °C



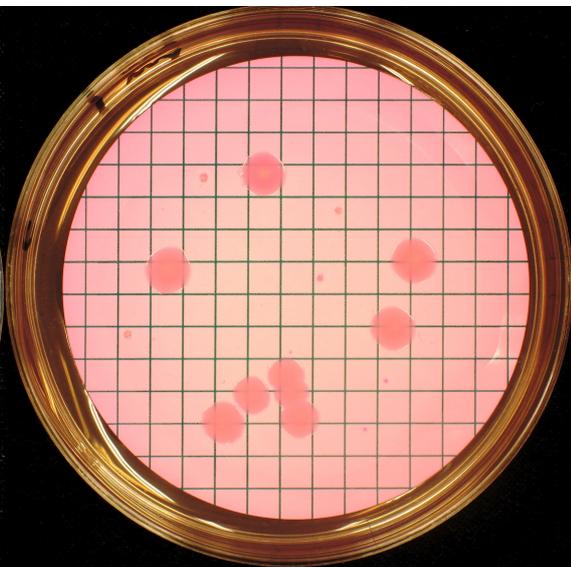
100 ml

Actinomycete Isolation Agar, 25 °C



1 ml, 7 days

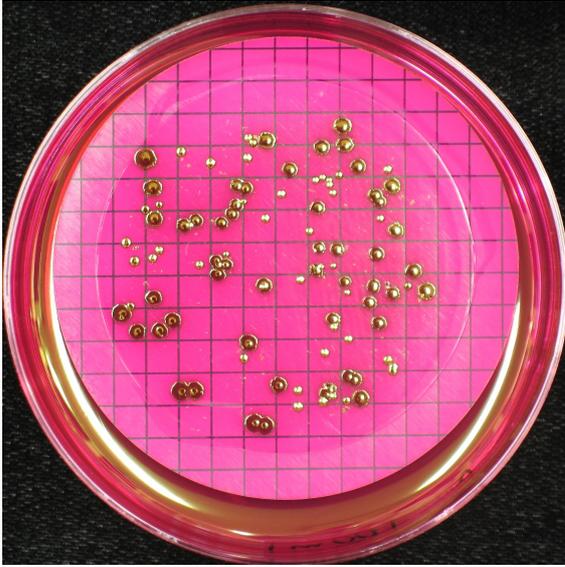
m-RBCC Agar, 25 °C



1 ml, 7 days

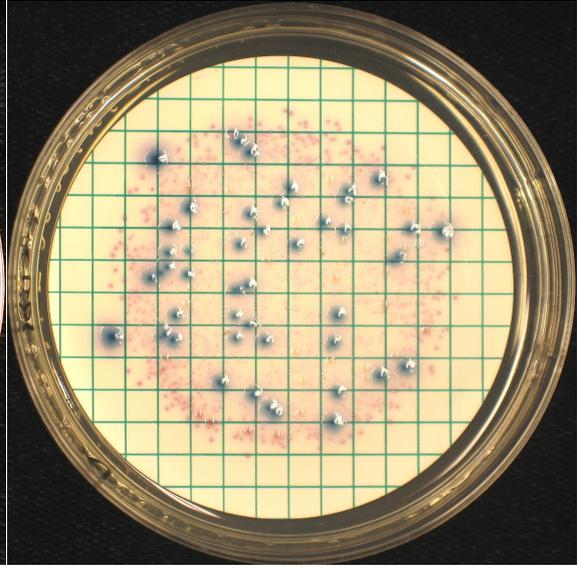
Mixture B

m-Endo Agar LES, 37 °C



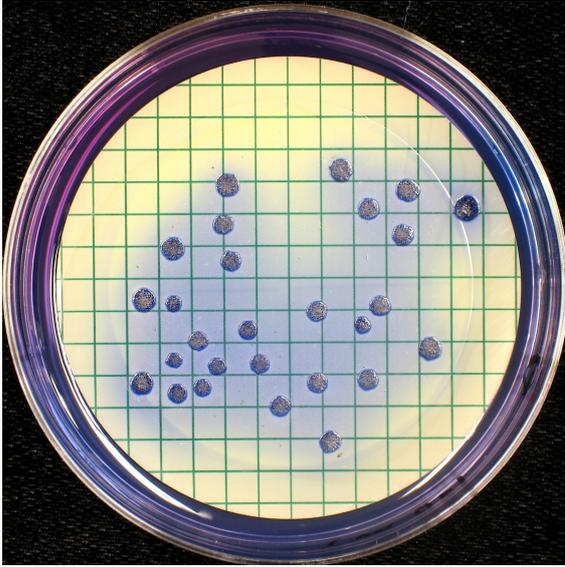
100 ml

Chromocult Coliform Agar, 37 °C



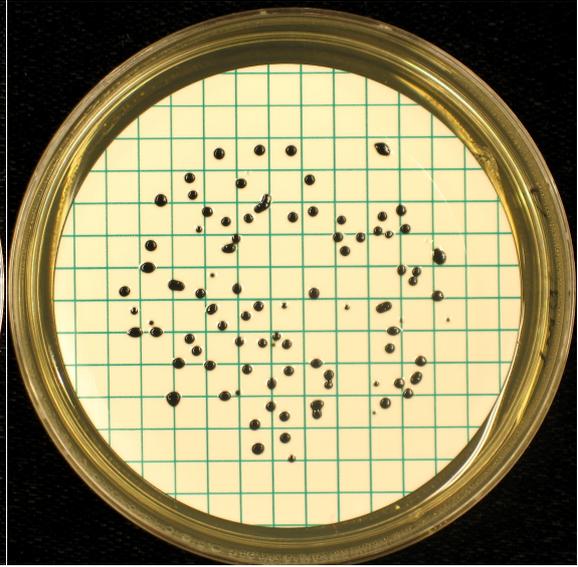
100 ml

m-FC Agar, 44 °C



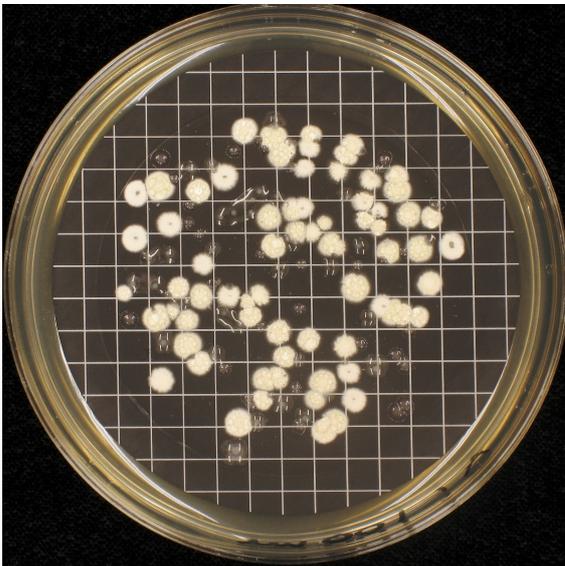
100 ml

m-TSC Agar, 44 °C



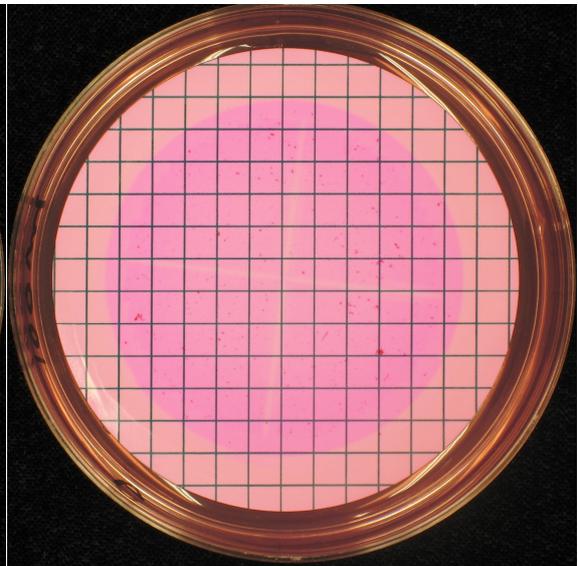
10 ml

Actinomycete Isolation Agar, 25 °C



100 ml, 7 days

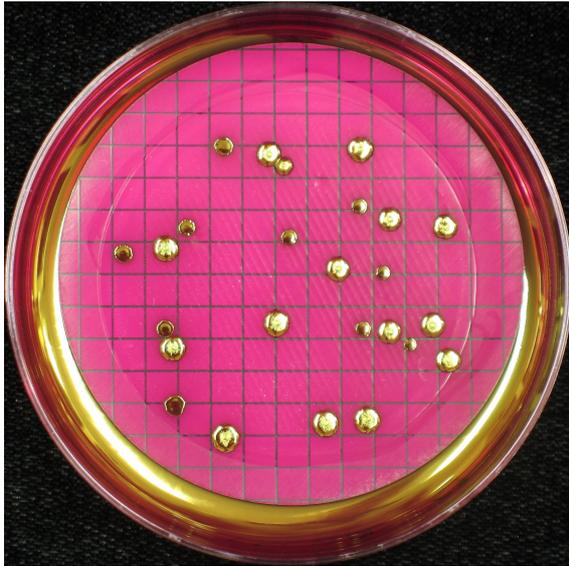
m-RBCC Agar, 25 °C



100 ml, 7 days

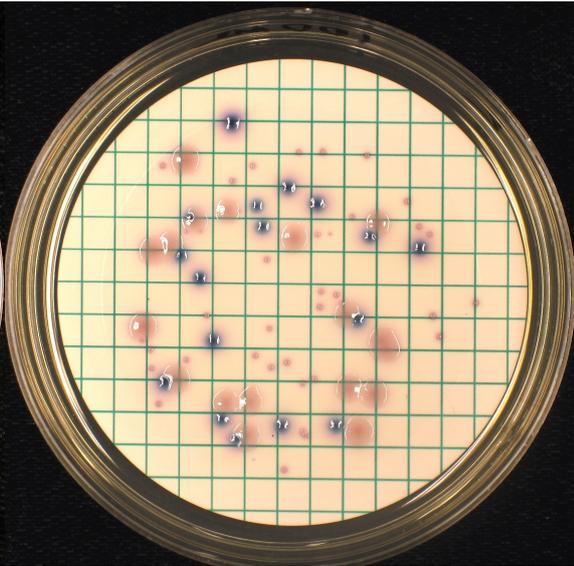
Mixture C

m-Endo Agar LES, 37 °C



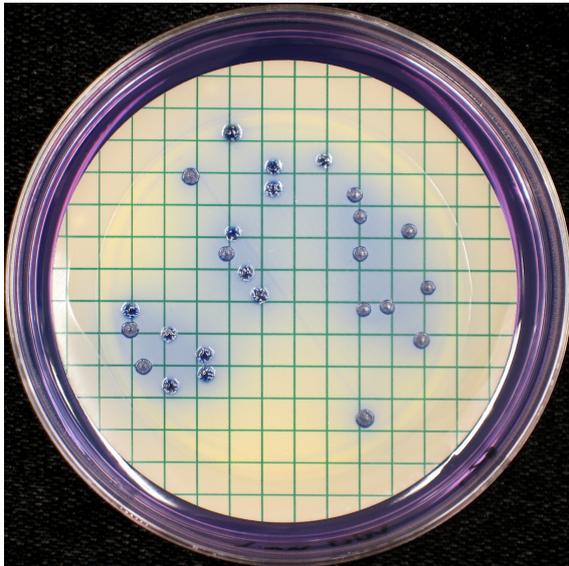
100 ml

Chromocult Coliform Agar, 37 °C



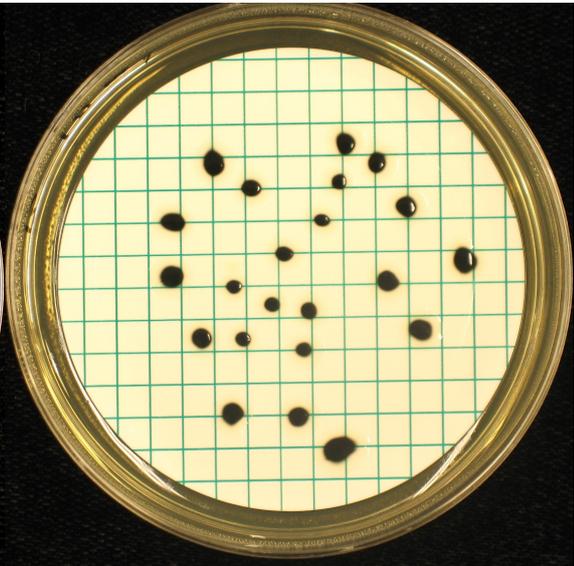
100 ml

m-FC Agar, 44 °C



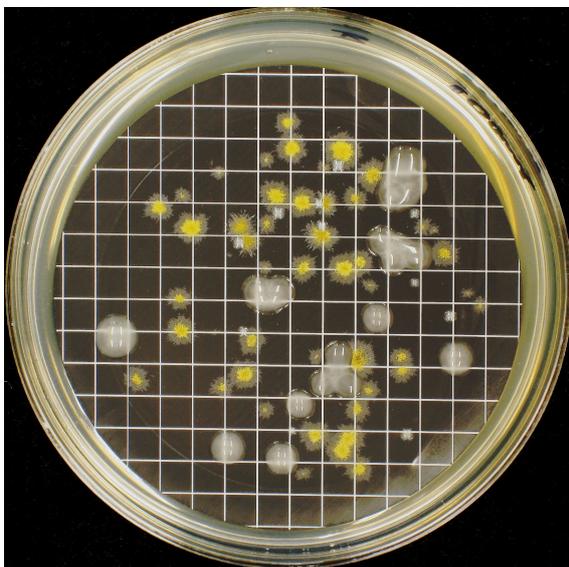
100 ml

m-TSC Agar, 44 °C



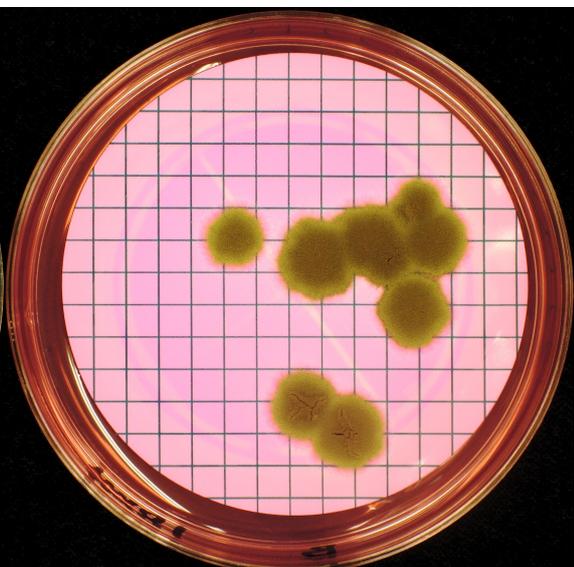
10 ml

Actinomycete Isolation Agar, 25 °C



100 ml, 7 days

m-RBCC Agar, 25 °C



10 ml, 7 days

PT reports published 2016

Proficiency Testing – Food Microbiology, January 2016, by Kirsi Mykkänen

Proficiency Testing – Drinking Water Microbiology, March 2016, by Tommy Šlapokas

Proficiency Testing – Food Microbiology, April 2016, by Jonas Ilbäck

Proficiency Testing – Drinking Water Microbiology, September 2016, by Tommy Šlapokas

Proficiency Testing – Food Microbiology, October 2017, by Jonas Ilbäck

PT reports published 2017

Proficiency Testing – Food Microbiology, January 2017, by Jonas Ilbäck

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

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

In a proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The laboratories report their results to the organiser that evaluates them and compiles them in a report.

The National Food Agency's PT program offers

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

For more information visit our website: www2.slv.se/absint

The National Food Agency's reference material

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

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