Drinking Water Microbiology March 2019

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Proficiency testing Drinking water Microbiology March 2019

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[®] (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 EN ISO 14189:2016)
- 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 *excluded*)
- Med median value (with outliers and false results *included*)
- 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
- 601 remarkably low result
- 278 remarkably high result or CV or many deviating results

Explanations to histograms with accepted and deviating results

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

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General information on results evaluation

The proficiency testing program organised by the 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. Here are reported method data for each parameter where differences are present or could be expected.

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

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in tables together with the group means etc. The mean and measure of dispersion are not shown for groups with 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 29 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 82 laboratories, 35 in Sweden, 42 in other Nordic countries (Faeroe Islands, Greenland and Åland included), 3 more from EU, 1 from the rest of Europe and one from outside Europe. Results were reported from 81 laboratories.

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

Microorganisms and parameters of analyses are also compiled in table 1. For the MF analyses the parameters *suspected* coliform and thermotolerant coliform bacteria could be reported. The results from suspected colonies are only used for interpretations and discussions, not for assessment.

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

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

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

Sample	Α			В			C		
Percentage of laboratories with 0 deviating results 1 deviating result 2 deviating results >2 deviating results	4% 1% 7%	88%		4% 1% 7% 88	3%		1% 1%	74%	
No. of evaluable results	510			509			508		
No. of deviating results $*$	15	(3%)		15 ((3 %)		25	(5%)	
Microorganisms	Escherichia coli Klebsiella pneur Kluyveromyces Phialophora ma Staphylococcus	monia marxia Ilorum	anus	Enterobacter aer Aeromonas hydro Clostridium perfi Issatchenkia orie Cladosporium cladosporioia	ophila ringen entalis	us	Escherichia col Citrobacter freu Clostridium bife Streptomyces sg Stenotrophomor maltophilia	ındii erment 5.	ans
Analysis	Target org.	F%	X%	Target org.	F%	X%	Target org.	F%	X%
Coliform bacteria (MF)	E. coli K. pneumoniae	0	0	E. aerogenes [A. hydrophila]	2	2	E. coli C. freundii	0	5
Susp. thermotolerant coliform bact. (MF)	E. coli K. pneumoniae	-	_	{E. aerogenes}	-	-	E. coli	-	-
E. coli (MF)	E. coli	8	3	_	0	-	E. coli	0	0
Coliform bacteria (rapid method)	E. coli K. pneumoniae	0	0	E. aerogenes	0	7	E. coli C. freundii	0	2
E. coli (rapid meth.)	E. coli	2	0	_	0	-	E. coli	0	3
Presumptive C. perfringens (MF)	_	0	—	C. perfringens	1	0	C. bifermentans	13	0
Clostridium perfringens (MF)	_	3	-	C. perfringens	3	0	[C. biferment.]	17	-
Actinomycetes (MF) 25 °C	_	3	_	_	3	_	Streptomyces sp.	0	0
Moulds (MF) 25 °C	Ph. malorum	3	0	C. cladosporioides	0	3	_	3	_
Yeasts (MF) 25 °C	K. marxianus	3	3	I. orientalis	0	3	—	5	_
Culturable micro- 22 °C organisms (total count), 3 days	S. cohnii (E. coli) (K. pneumoniae)	0	3	A. hydrophila E. aerogenes	3	3	S. maltophilia (E. coli) (C. freundii)	0	5

* In total 29 of 81 laboratories (36 %) reported at least one deviating result

- Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

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

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

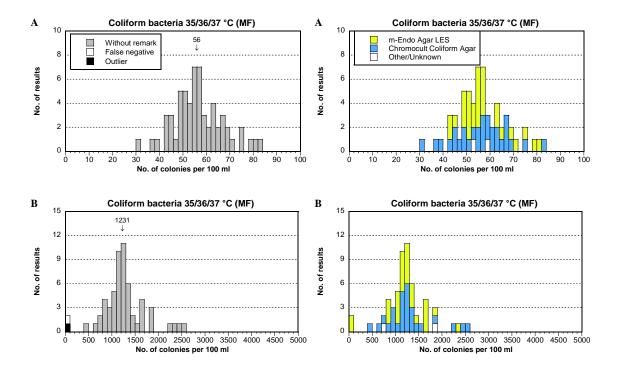
Coliform bacteria (MF)

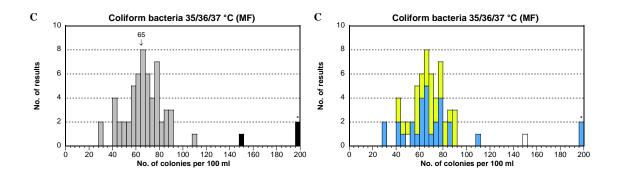
One of the two laboratories in the group Other/Unknown in the table has used Tryptone Glucose Extract agar (TGE) and incubated 7 days in room temperature. The other laboratory stated that they used Brilliance E. coli/Coliform Selective Agar, but yet claimed the use of ISO 9308-1:2014.

From the table it is clear that this time approximately the same number of laboratories used CCA and LES. The proportion that used CCA has continued to increase since the standard EN ISO 9308-1 from 2014 was issued. The use of LTTC from the previous edition of that standard has practically ceased.

This time the results for LES and CCA are approximately equal. LES has previously often given somewhat higher average results than CCA. Also for other method groups no obvious differences could be seen this time. In total five different coliform bacteria, including *E. coli*, were present in the samples.

Medium	Ν			Α						В						С			
wiedium	IN	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	60	60	56	10	0	0	0	57	1231	16	1	1	0	57	65	12	0	0	3
m-Endo Agar LES	29	29	56	9	0	0	0	27	1242	13	1	1	0	29	67	10	0	0	0
Chromocult C Agar	29	29	55	11	0	0	0	28	1219	18	0	0	0	27	63	14	0	0	2
Other/Unknown	2	2	_	_	0	0	0	2	_	_	0	0	0	1	_	_	0	0	1





Sample A

- A strain of *Escherichia coli* and a strain of *Klebsiella pneumoniae* were included. They appeared with for coliform bacteria typical colonies on the MF media at 37 °C, a metallic sheen on LES and blue and pinkish red, respectively, on CCA.
- The distribution of the results was good with very small to small dispersion (CV; see page 29). No deviating results were present.

Sample B

- No *E. coli* but the coliform bacteria, *Enterobacter aerogenes*, was present. This strain together with a strain of *Aeromonas hydrophila* appeared with, for coliform bacteria, typical colonies at 37 °C, i.e. with metallic sheen on LES and pinkish on CCA.
- The distribution of the accepted results was fairly good and the dispersion small. One false negative result and 1 low outlier were present.
- A. hydrophila was a false positive strain but could be removed after confirmation with oxidase test because it is oxidase positive. The results for suspected coliform bacteria and coliform bacteria were identical in 10 laboratories. The distribution of the results, indicates that *A. hydrophila* probably in most cases was not even included among the suspected ones. Individual laboratories might, however, have missed to exclude those colonies after confirmation.

Sample C

- One strain each of *E. coli* and *C. freundii* were present as coliform bacteria. They appear with for coliform bacteria typical colonies on the MF media at 37 °C, a typical metallic sheen on LES and blue and pink, respectively, on CCA.
- Three high outliers were present, out of which 2 by unknown reason were very high. The distribution was good and the dispersion small.

Suspected thermotolerant coliform bacteria (MF)

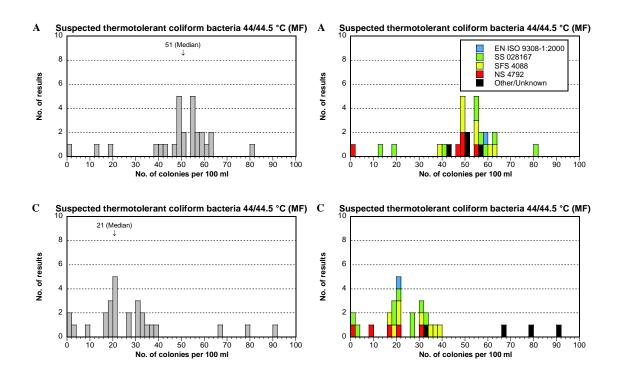
No evaluation in relation to performance is done for what is called suspected (not confirmed) colonies of a parameter. Therefore, no identification of outliers is done. The *medians* are then more robust than the means and are given in the table and in histograms. **Thus, the parameter is not included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

Previously, the two most used growth media have been m-FC and LTTC. The incubation temperature is 44 or 44.5 °C. Only one result for LTTC was reported and was not deviating from the others for m-FC. Two of the results for Other/Unknown are from Icelandic laboratories that in principle perform the analyses in accordance to "ISO 9308-1:1990, modified". For drinking water, the primary incubation is at 37 °C, and 44 °C is used only for confirmation. Hence, this is not an analysis of suspected thermotolerant coliform bacteria, which probably explains their high results of sample B and C. The same reasoning is valid for the other two results in the group Other/Unknown that also can be considered obtained by a wrong method.

Standard Mathad	N			Α						В						С			
Standard, Method	Ν	n	Med	CV	F	<	>	n	Med	CV	F	<	$^{\prime}$	n	Med	CV	F	<	>
Total	27	27	51	-	-	—	-	27	0	-	-	-	-	27	21	_	—	—	—
ISO 9308-1:2000	1	1	_	_	_	_	_	1	_	_	_	_	-	1	_	_	_	_	_
SS 028167	9	9	55	_	_	_	_	9	400	_	_	_	_	9	20	_	_	_	_
SFS 4088	8	8	51	_	_	_	_	8	0	_	_	_	-	8	26	_	_	_	_
NS 4792	5	5	48	_	_	_	_	5	0	_	_	_	-	5	16	_	_	_	_
Other/Unknown	4	4*	51	_	_	_	_	4*	556	_	_	_	—	4*	72	_	_	_	—

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

^{*} Median are given for comparison despite few results



These laboratories state that they specifically don't test for suspected thermotolerant coliform bacteria and probably instead have presented results from incubation at 36 ± 2 °C. The strains of *E. aerogenes* and *C. freundii* in sample B and C respectively, seem to be included in the results from the Icelandic and one of the two other laboratories in the group Other/Unknown. These strains may sometimes grow at near 44 °C but usually not at 44 °C, as they are not thermotolerant.

Sample A

- Both the strain of *E. coli* and the strain of *K. pneumoniae* grow as typical suspected thermotolerant coliform bacteria with blue colonies on m-FC agar at 44/44.5 °C.
- The distribution of the 27 results was good in general.

Sample B

- One strain of *E. aerogenes* together with a strain of *A. hydrophila* appear on media for coliform bacteria at 35-37 °C. The strain of *A. hydrophila* does not grow at 44 °C while the strain of *E. aerogenes* sometimes may appear with small blue colonies on m-FC, in particular if the temperature does not reach 44 °C.

Sample C

- Two coliform bacteria were included in the mixture, of which the *E. coli* strain appears as a typical suspected thermotolerant coliform bacterium at 44 °C, meaning blue colonies on m-FC. The strain of *C. freundii* normally doesn't grow on agar media at 44 °C.
- The distribution of the 27 results was good in general. The 3 highest results could be seen as outliers but, as mentioned previously, they don't originate from the analysis of strictly thermotolerant organisms.
- The strain of *E. coli* is gas negative. Gas production at 44/44.5 °C is in some standards a criterion for a strain to be included among the thermotolerant coliform bacteria. If this criterion has been used also when reporting suspected thermotolerant coliform bacteria which is not in the definition of the parameter it is probable that the colonies from *E. coli* have not been reckoned.

Escherichia coli (MF)

To identify and quantify *E. coli*, confirmation is required when colonies are isolated from the primary cultivation media LES, LTTC and m-FC. Depending on the method, test of indole production and/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. Here are the results instead grouped by standard. This time there were no results reported for LTTC based on the standard ISO 9308-1:2000. For ISO 9308-1:2014 the incubation is 36 ± 2 °C on CCA. For the standards from the Nordic countries (SS and SFS) the majority of the results are from 36 ± 2 °C

on LES but some are also from 44/44.5 °C on m-FC. Actually, only one Finnish laboratory has stated the standard SFS 4088 (m-FC) instead of SFS 3016 for the analysis of *E. coli*.

The results are additionally grouped based on the reported incubation temperature.

When all results are compared, there is in principle no differences between the different standards or incubation temperatures for any sample. For sample C the result at 44/44.5 °C might be somewhat lower. Neither the results nor the dispersion (CV) is this time different for CCA compared to LES.

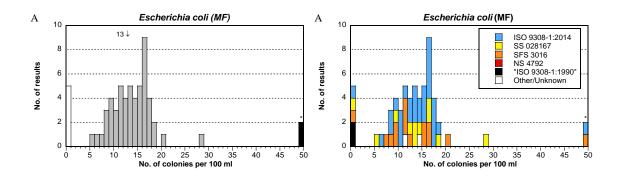
All results

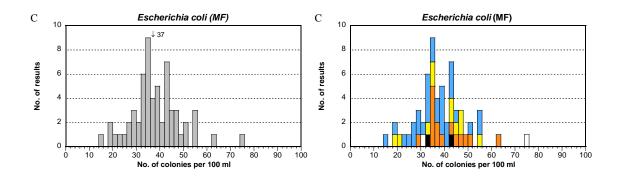
Origin & Standard	Ν			Α						В						С			
Origin &Standard	IN	n	Mv	CV	F	<	$^{<}$	n	Mv	CV	F	<	$^{<}$	n	Mv	CV	F	<	>
Total	60	53	13	15	5	0	2	60	0	_	0	_	Ι	60	37	14	0	0	0
<u>Colony origin</u>																			
36 ± 2 °C	45	42	13	10	2	0	1	45	0	_	0	_	_	45	37	16	0	0	0
44/44.5 °C	5	3	13	4	1	0	1	5	0	_	0	_	_	5	33	14	0	0	0
36 ± 2 & 44/44.5 °C	9	7	12	14	2	0	0	9	0	_	0	_	_	9	38	7	0	0	0
Other/Unknown	1	1	_	-	0	0	0	1	0	-	0	—	_	1	_	_	0	0	0
<u>Standard</u>																			
ISO 9308-1:2014	30	28	13	13	1	0	1	30	0	_	0	_	_	30	35	14	0	0	0
SS 028167	11	10	14	21	1	0	0	11	0	_	0	_	_	11	37	16	0	0	0
SFS 3016 (4088)	15	13	12	16	1	0	1	15	0	_	0	_	_	15	40	10	0	0	0
NS 4792	0	0	_	_	_	_	_	0	_	_	_	_	_	0	_	_	_	_	_
"ISO 9308-1:1990"	2	0	_	-	2	0	0	2	0	-	0	_	_	2	_	_	0	0	0
Other/Unknown	2	2	_	_	0	0	0	2	0	_	0	_	_	2	_	_	0	0	0

Results from the analysis of coliform bacteria MF at 36 ± 2 °C

Madium	N			Α						В						С			
Medium	Ν	n	Mv	CV	F	<	<	n	Mv	CV	F	<	<	n	Mv	CV	F	<	>
Total	48 [#]	45	13	16	3	0	0	4 8	0	_	0	_	Ι	<i>48</i>	38	14	0	0	0
m-Endo Agar LES	17	15	13	21	2	0	0	17	0	-	0	_	Ι	17	40	13	0	0	0
Lactose TTC Agar	0	0	_	_	_	_	_	0	_	_	_	_	_	0	_	_	_	_	_
Chromocult C Agar	30	29	13	13	1	0	0	30	0	_	0	_	—	30	36	13	0	0	0
Other/Unknown	1	1	_	_	0	0	0	1	0	_	0	_	_	1	_	_	0	0	0

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





Sample A

- One typical strain of *E. coli* was present together with another thermotolerant coliform bacterium, *K. pneumoniae*. The latter is indole negative, and has no activity of β -glucuronidase. Thus, it cannot be taken for *E. coli* after confirmation.
- The distribution of the results was good and the dispersion small (se p. 29) except the deviating results. Five false negative results and 2 high outliers were present.
- The real reason to the zero results is not clear. For example, both the results based on the modified "ISO9308-1:1990" were zero but even other standards occasionally gave such results. In all cases the confirmation seem to be the cause, as there are other results present in the presumptive analyses.

Sample B

- No *E. coli* was included but another coliform bacterium, *E. aerogenes*, was present together with another coliform-like bacterium, *A. hydrophila*. The latter is oxidase positive. *E. aerogenes* is indole negative and has no activity of β -glucuronidase. Thus, even that strain can't be taken for *E. coli* after confirmation.
- No false positive results were reported.

Sample C

- A strain of *E. coli* was included together with another coliform bacterium, *C. freundii*. Sometimes small blue colonies of *C. freundii* can appear on m-FC at near 44 °C. The colony appearance for *E. coli* is typical on LES and m-FC, which are based on lactose fermentation as well as on the enzyme-based chromogenic medium CCA. The colonies of *E. coli* on CCA are typical blue and thus they are confirmed. The colonies of *C. freundii* are pink. Confirmation for *E. coli* is not required from CCA but is necessary to discern *E. coli* from other coliform bacteria for colonies picked from LES and m-FC. The strain is indole positive and show distinct β -glucuronidase activity.
- The distribution of the results was good and the dispersion (CV) small. No deviating results were present.
- The strain of *E. coli* is not producing gas in lactose broth at 44 °C. If gas production is a decisive criterion for a laboratory to detect *E. coli*, they should have reported a zero result. However, no zero results were reported, indicating that gas production was not decisive.

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

The rapid method used for both these parameters was exclusively Colilert[®] Quanti-Tray[®] from the manufacturer IDEXX Inc. with incubation at 35, 36 or 37 °C. Out of the about 60 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 wells (a few of which, probably incorrectly, have reported 96 wells). The laboratories often analysed both diluted and undiluted samples. Yellow wells (ONPG positive; 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 is usually also small. In principle, no such differences can be seen this time, neither for coliform bacteria nor for *E. coli*. Not even the result for the group (the laboratory) with "24 hours" was this time lower than the others, as has previously been the case.

There is nothing in the evaluation that indicates problem with interpretation of the results.

In substian time	N			Α						В						С			
Incubation time	IN	n	Mv	CV	F	<	<	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	61	61	62	10	0	0	0	57	1276	10	0	4	0	60	69	12	0	1	0
(18 –) 20 hours	33	33	64	9	0	0	0	31	1307	11	0	2	0	33	68	11	0	0	0
(18 –) 22 hours	26	26	61	10	0	0	0	24	1229	7	0	2	0	25	71	13	0	1	0
18 - 24 hours	1	1*	57	_	0	0	0	1*	1246	_	0	0	0	1*	78	_	0	0	0
24 hours*	1	1*	64	-	0	0	0	1*	1500	-	0	0	0	1*	66	-	0	0	0

Coliform bacteria, Rapid method with MPN

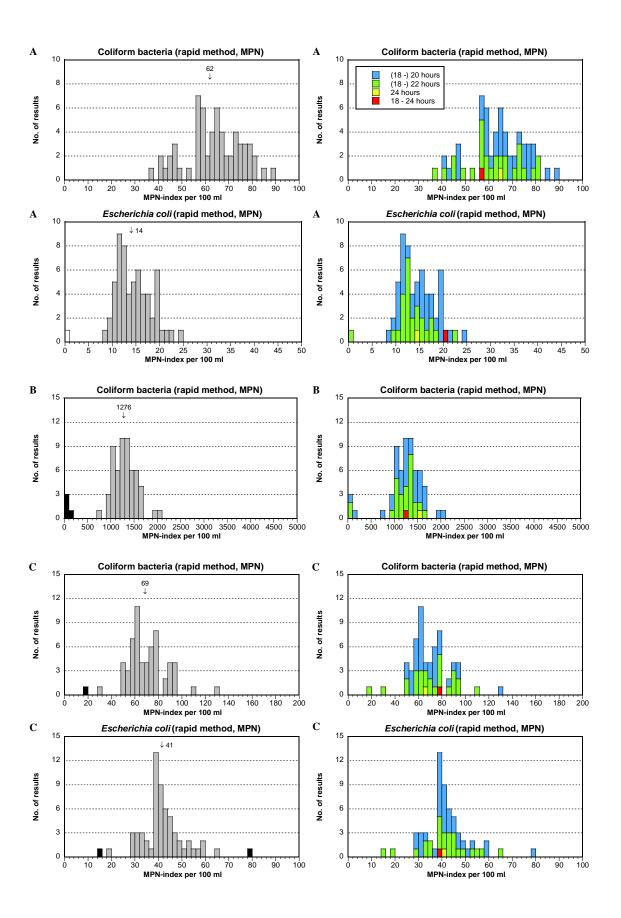
Е.	coli,	Rapid	method	with	MPN	
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Incubation time	Ν			Α						В						С			
incubation time	IN	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	<
Total, Rapid meth.	61	60	14	13	1	0	0	61	0	_	0	_	-	59	41	10	0	1	1
(18 –) 20 hours	34	34	14	14	0	0	0	34	0	_	0	_	Ι	33	40	9	0	0	1
(18 –) 22 hours	25	24	13	11	1	0	0	25	0	_	0	_	_	24	41	12	0	1	0
21 – 24 hours	1	1*	20	_	0	0	0	1	0	_	0	_	_	1*	39	_	0	0	0
24 hours*	1	1*	14	_	0	0	0	1	0	_	0	_	_	1*	40	-	0	0	0

* Mean value is given for comparison despite few results

Sample A

- 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.
- Only the strain of *E. coli* has the enzyme β -glucuronidase and is detected as *E. coli*.



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- The distributions of the results were good and the dispersions (CV) were very small to small (se p. 29). One false negative result for *E. coli* was the only deviating result.
- The average for coliform bacteria with this rapid method were somewhat higher than with the MF method but about equal for *E. coli* (compare p. 6 and 10).

Sample B

- The strain of *E. aerogenes* is the only coliform bacterium that grows in the medium and has the enzyme β -galactosidase. Therefore, it is detected as a coliform bacterium by methods based on this enzyme (ONPG positive) e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate.
- The strain of *E. aerogenes* lacks the enzyme β -glucuronidase and is thus not detected as *E. coli*.
- The distribution of the results for coliform bacteria was good with small dispersion. Four low outliers were reported.
- The average for coliform bacteria was barely higher than with the MF methods.

Sample C

- The mixture contained the two coliform bacteria *E. coli* and *C. freundii*. Both of them possess β -galactosidase (ONPG positive) and are detected as coliform bacteria.
- Only the strain of *E. coli* has the enzyme β -glucuronidase and is detected as *E. coli*.
- The distributions of the results were good with small dispersions. One low outlier was reported for coliform bacteria and 1 low and 1 high for *E. coli*.
- The averages for both coliform bacteria and *E. coli* were approximately the same as for the MF methods.

Presumptive and confirmed *Clostridium perfringens* (MF)

The analysis of *Clostridium perfringens* has previously been performed differently in different countries and laboratories. The parameter to be analysed is the sum of spores and vegetative cells of *C. perfringens*. In Sweden presumptive *C. perfringens* are accepted, which is why that parameter is presented separately.

No international standard was stated as reference method in the European Drinking Water Directive from 1998 [4]. A specific method was instead explicitly included into the directive, the use of m-CP Agar incubated at 44 °C. The method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*.

Due to the hesitation in many countries to use that method, the use of a standard still under process (ISO/CD 6461-2:2002-12-20; CD = Committee Draft), based on TSC agar (TSC), was accepted as an alternative by the responsible group under the EU Commission until a finished standard was available. Adjustments in the draft approved 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 based on TSC 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 tested only 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 have been taken into use no later than in October 2017 within EU, after being implemented in the national legislations. The CD version as well as m-CP agar are not valid for use in official drinking water monitoring after that date.

In the spring 2018 still 24 % of the laboratories used any of the old methods. This figure has now decreased to 18 % (10 out of 56 laboratories). Only one laboratory has this time stated the use of the m-CP for *Clostridium perfringens*. That result was not different from those for other methods.

For the two methods with TSC no difference can be seen in sample B. Only for presumptive *C. perfringens* in sample C, a slight difference between the new and the old method can be suspected. As it pertains a strain of *Clostridium bifermentans* that generally is causing very varying results, the difference is nothing to consider.

Standard/Method	N #			Α					В						С			
Stanuaru/Wiethou	IN	n	Mv	CV	F	< >	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	56	47	0	-	0		46	29	17	1	0	0	41	1204	45	6	0	0
(EN) ISO 14189	46	39	0	_	0	 	38	28	18	1	0	0	33	1285	45	6	0	0
ISO/CD 6461-2:2002	9	8	0	_	0		8	31	11	0	0	0	8	898	43	0	0	0
m-CP agar, EU-direct.	1	0	_	_	_		0	_	_	_	_	_	0	-	-	_	_	_

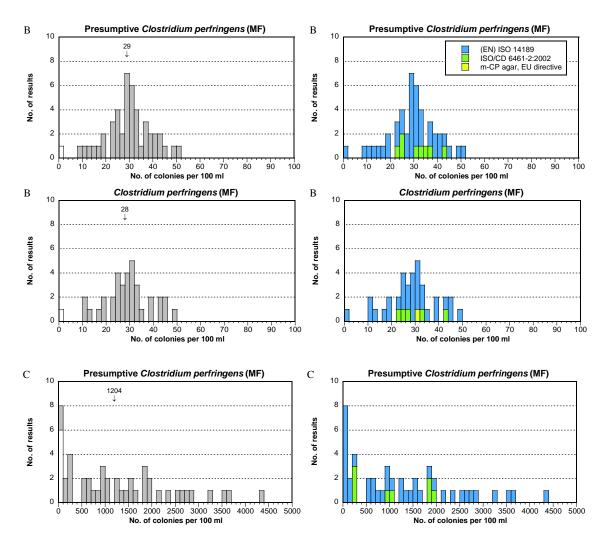
Presumptive Clostridium perfringens MF

Clostridium perfringens MF

Standard/Method	N #			Α					В						С		
Standard/Method	IN	n	Mv	CV	F	< >	n	Mv	CV	F	<	>	n	Mv	CV	F	< >
Total	56	29	0	_	1		35	28	17	1	0	0	35	0	-	1	
(EN) ISO 14189	46	24	0	_	1		29	28	19	1	0	0	29	0	_	1	
ISO/CD 6461-2:2002	9	4	0	_	0		5	29	13	0	0	0	5	0	_	0	
m-CP agar, EU-direct.	1	1	0	_	0		1*	30	_	0	0	0	1	0	-	0	

* Mean value is given for comparison despite few results

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



Sample A

- No presumptive *C. perfringens* was included. Yet, 1 false positive result was present for *C. perfringens*.

Sample B

- 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.

- One false negative result each was present in the presumptive test and for *C*. *perfringens*.
- The distribution of the results was, as in the previous spring, unusually good for both presumptive and confirmed *C. perfringens*, without the earlier occurring tail of low results. The reason is probably that this time only one result was present from m-CP agar that earlier have given considerably lower results than TSC. The dispersion (CV) was even this time not higher than for other parameters, but small (see p. 29).

Sample C

- No *C. perfringens* was included but instead a strain of *C. bifermentans*. The strain appeared on TSC with small, black to almost transparent presumptive colonies. Confirmation reveals that they are not from *C. perfringens*.
- There is no tendency to Poisson distribution of the presumptive results as there are many low values. The dispersion (CV) was very large implying that no outliers could be identified. Six false negative results were obtained.
- In the analyses of *C. perfringens* 1 false positive result was present.

Moulds and yeasts (MF)

Out of the 39 laboratories that analysed moulds and yeasts, 29 reported that they used the Swedish standard SS 028192. Besides Sweden it is used in Finland under their own national designations SFS 5507. Sometimes it is modified regarding media composition as for example dichloran (DRBC) is used.

Various names are reported for the media linked to the use of SS 028192 and SFS 5507. These are: Cooke Rose Bengal Agar base, Rose Bengal Agar, Rose Bengal Chloramphenicol Agar and Dichloran Rose Bengal Chloramphenicol Agar (DRBC). According to the original 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. Both of them are at least used by 17 of 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) and the six laboratories from various countries stating DRBC in conjunction with SS 028192 or SFS 5507 – or in one case "Standard methods" [5] – (DRBC "Water").

Two Norwegian laboratories instead stated NMKL 98:2005, modified to be used with DRBC. This comprises the group DRBC "Food" in the tables. Three Finnish laboratories used Malt Extract Agar; one in conjunction with NMKL 98:2005 and the remaining ones with other non-water methods. These 3 laboratories are placed in the group ME. Two Finnish laboratories using Oxytetracycline Glucose Extract Agar based on other methods/standards are placed in the group OGYE. In several of these groups there are so few results that it is not meaningful to discuss possible differences. But the mean values are still given for comparison.

DRBC "Water" seems to give somewhat higher results for both moulds and yeasts in sample A compared to RBC. In all three cases a selective substance (dichloran, chloramphenicol or streptomycin) has been added to ME, making it selective. Yet, the average results seem even there to be somewhat higher compared to most other media in sample A.

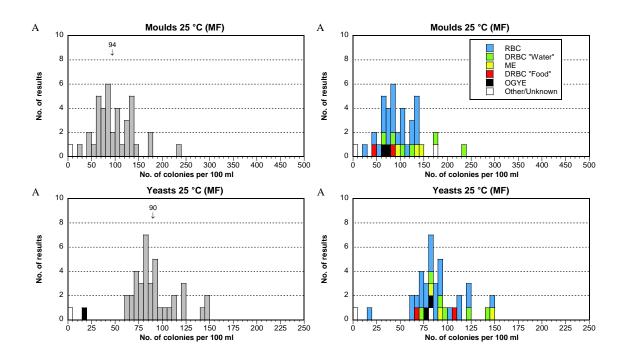
Ston doud/Mothod	N			Α						B						С			
Standard/Method	IN	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	<	n	Mv	CV	F	<	>
Total	39	38	94	21	1	0	0	38	6	41	0	0	1	38	0	_	1	-	-
RBC	24	24	87	19	0	0	0	24	6	39	0	0	0	23	0	_	1	_	-
DRBC "Water"	6	6	122	_	0	0	0	5	7	20	0	0	1	6	0	_	0	_	_
ME	3	3*	122	_	0	0	0	3*	9	_	0	0	0	3	0	_	0	_	—
DRBC "Food"	2	2*	62	_	0	0	0	2^*	8	_	0	0	0	2	0	_	0	_	_
OGYE	2	2*	68	_	0	0	0	2^*	8	_	0	0	0	2	0	_	0	_	_
Other/Unknown	2	1	_	_	1	0	0	2^*	0	_	0	0	0	2	0	_	0	_	_

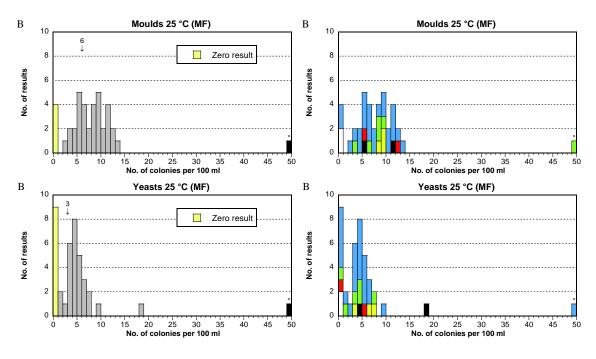
Moulds MF

Yeasts MF

Ston dond/Mothod	N			Α						В						С		
Standard/Method	IN	n	Mv	CV	F	<	<	n	Mv	CV	F	<	<	n	Mv	CV	F	< >
Total	39	37	90	12	1	1	0	38	3	65	0	0	1	37	0	_	2	
RBC	24	23	88	11	0	1	0	23	2	59	0	0	1	22	0	_	2	
DRBC "Water"	6	6	98	13	0	0	0	6	2	60	0	0	0	6	0	_	0	
ME	3	3*	104	_	0	0	0	3*	5	_	0	0	0	3	0	_	0	
DRBC "Food"	2	2*	84	_	0	0	0	2^*	1	_	0	0	0	2	0	_	0	
OGYE	2	2*	79	_	0	0	0	2^*	10	_	0	0	0	2	0	_	0	
Other/Unknown	2	1	_	_	1	0	0	2^*	0	_	0	0	0	2	0	_	0	

* Mean value is given for comparison despite few results





Sample A

- The mould *Phialophora malorum* and the yeast *Kluyveromyces marxianus* were included in approximately the same concentration. No apparent problem could be seen and the distributions of the results were good with a medium and small dispersion (CV), respectively, for moulds and yeasts.
- One false negative result was present for the moulds.
- One false negative result and 1 low outlier were present for the yeasts.

Sample B

- The mould *Cladosporium cladosporioides* and the yeast *Issatchenkia orientalis* were included in low concentrations, lowest for the yeasts. With the exception of the many zero results, the result distributions were good, even though the relative dispersions (CV) for the analyses were large to very large due to the low concentrations.
- One high outlier each was present for the two parameters. Further, there were 4 false negative mould results present as well as 9 false negative yeast results.
- The zero results at least for yeasts are probably caused by mere chance due to the low concentrations. However, the zero results are here accepted both for moulds and yeasts and are not considered as false negative results.

Sample C

- Neither moulds nor yeasts were included. Yet, 1 false positive result was reported for moulds and 2 false positive results for yeasts. In one case for yeasts, where only 1 colony was found, it can be a contamination from the laboratory air. Such results should not be seen as false positive ones.

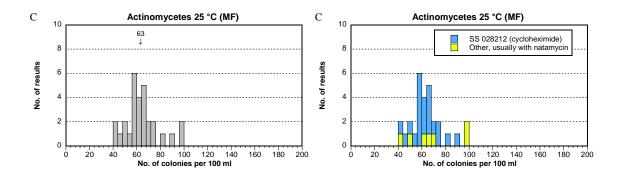
Actinomycetes (MF)

The analysis of actinomycetes is included because it is a prescribed method for drinking water monitoring 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. Six of these have stated that they used natamycin as the selective substance instead of cycloheximide. The last laboratory also didn't use cycloheximide, but did not specify beyond "Other" what they used. Probably they have used natamycin as well. 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 Finnish laboratories checked the results after 7 and 14 days.

The averages of the two groups in sample C is approximately equal but the dispersion (CV) is twice as large for the group Other compared to the group ACTA. This pertains to the strain and sample included here but cannot be considered to be generally valid. The large dispersion for the group Other is probably caused by variations among the media used.

All results

Madium/Standard	N			Α					В					С			
Medium/Standard	IN	n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	<	>
Total	29	28	0	_	1		28	0	_	1		29	63	11	0	0	0
ACTA (SS 028212)	22	21	0	_	1		21	0	_	1		22	62	9	0	0	0
Other	7	7	0	_	0		7	0	_	0		7	67	17	0	0	0



Samples A and B

- These samples contained no actinomycetes. One false positive result from each of the samples were reported.

Sample C

- One actinomycete within the group *Streptomyces* sp. was included. The distribution of the results was good and the average dispersion small.
- There was no deviating result present.

Culturable microorganisms 22 °C, 3 days

Seventy-six of the 78 laboratories performing the analysis reported EN ISO 6222:1999 as method, which prescribes the use of Yeast extract Agar (YeA). Seven laboratories used Plate Count Agar instead but they have simultaneously stated the use of EN ISO 6222:1999. One laboratory used YeA in conjunction with "Standard methods" [5]. The majority of the laboratories have claimed counting both bacteria colonies as well as fungal colonies while eleven reported that they don't count fungi. Three others state that they count yeasts but not moulds.

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

It is difficult to find any consistent method difference. In sample A, Plate Count Agar seems also this time to give lower result than YeA instead of higher, as sometimes earlier. However, the dispersion (CV) is higher for these results. No general difference was seen in relation to magnification. There might be a tendency to higher results with the highest magnification, but it is weak. The culturable microorganisms were easy to count in all samples. There were no small colonies present that could be difficult to distinguish. This explains why there were no difference when different magnification was used for counting.

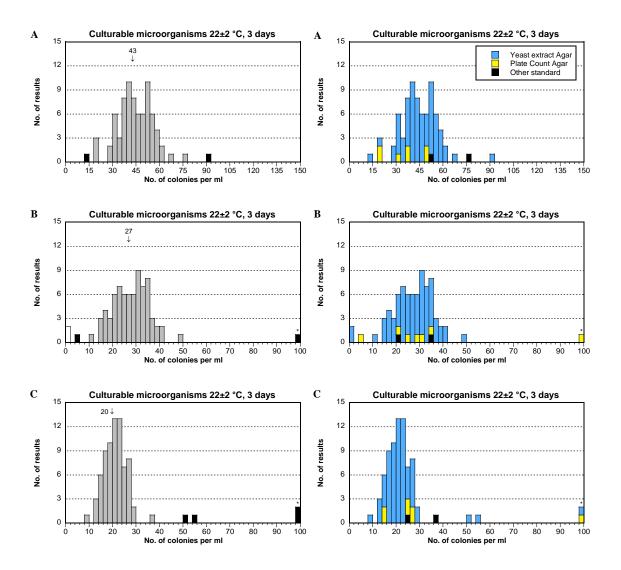
The distributions were good for all samples and the dispersions were small (see p. 29). Some deviating results were reported for each sample.

Choup of populta	Ν			Α						В						С			
Group of results	IN	n	Mv	CV	F	<	$^{<}$	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	78	76	43	12	0	1	1	74	27	14	2	1	1	73	20	11	0	0	4
EN ISO 6222	76	74	43	12	0	1	1	72	27	14	2	1	1	71	20	11	0	0	4
<u>Medium</u>																			
Yeast extract Agar	69	67	44	11	0	1	1	67	27	14	2	0	0	65	20	10	0	0	3
Plate Count Agar	7	7	34	18	0	0	0	5	27	11	0	1	1	6	22	13	0	0	1
Other/Unknown	0	0	_	-	_	_	_	0	_	_	_	_	_	0	_	_	_	_	-
Magnification																			
None	18	18	41	11	0	0	0	18	28	13	0	0	0	17	19	14	0	0	1
1,1–4,9×	29	28	43	15	0	1	0	27	24	15	0	1	1	26	20	9	0	0	3
5–11,9×	29	28	45	10	0	0	1	27	29	12	2	0	0	28	21	10	0	0	0
>12×	0	0	_	_	_	_	_	0	_	_	_	_	_	0	_	_	_	_	_
Other method	2	2*	64	_	0	0	0	2*	27	_	0	0	0	2*	30	_	0	0	0

* Mean value is given for comparison despite few results

Sample A

- It is mainly colonies of *Staphylococcus cohnii* that are growing but the other bacteria and the yeasts may also appear with individual colonies.
- The distribution of the results was good, but with 1 low and 1 high outlier.



Sample B

- The colonies that appear on the plates are practically only from *E. aerogenes* and *A. hydrophila*.
- The distribution of the results was good but with 2 false negative results and 1 low and 1 high outlier.

Sample C

- The colonies mainly consist of the strain of *Stenotrophomonas maltophilia* but at least also from the actinomycete individual colonies may also appear.
- The distribution of the results was good with small dispersion, with exclusion of the 4 high outliers.

Outcome of the results and laboratory assessment

General information about reported results

The distributions of results for the respective analysis are shown in 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.

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

Eight laboratories have more than one deviating result. 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 neither vials nor individual results for a parameter. Some laboratories may have performed incorrect calculations from their colony readings to the final concentrations.

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

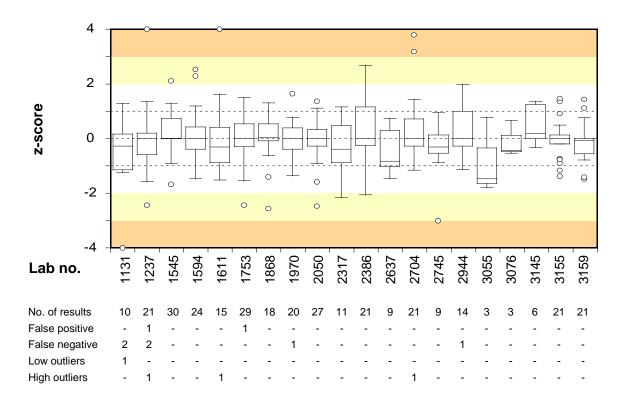
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are reported in annex B but not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control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

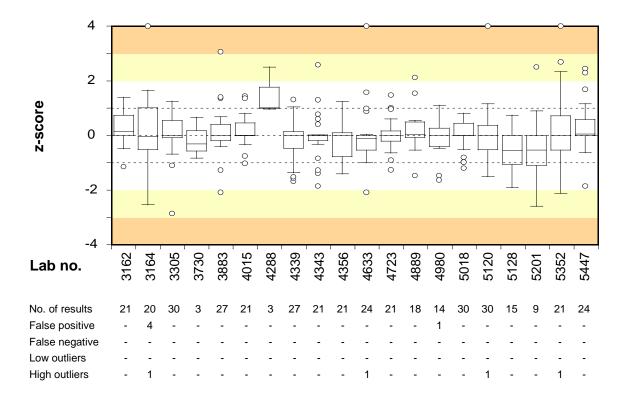
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

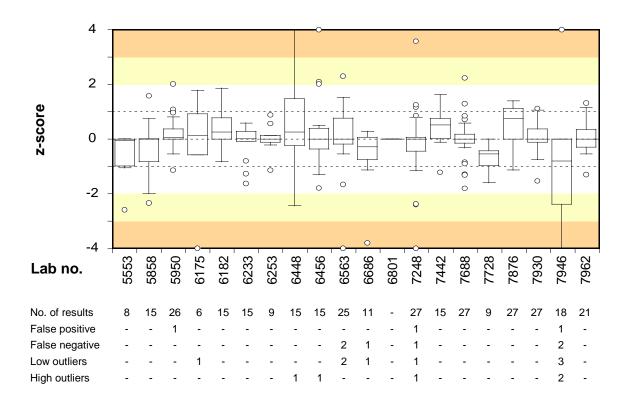
Box plots and numbers of deviating results for each participating laboratory

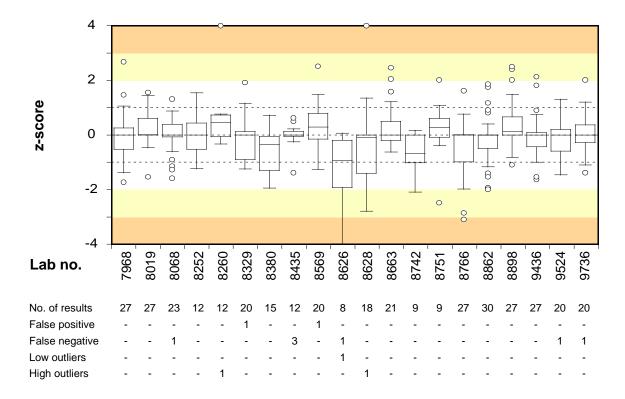
- *z*-scores are calculated from the formula z = (x mv) / s (see annex A).
- A correct result "zero" will get z = 0 when there is no target organism present.
- False results do not generate z-scores and are not included in 'No. of results'.
- The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as the rest of the results for each parameter.
- *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 for technical reasons shown when a result is to a certain degree deviating* from the rest. This alone does not mean it is an outlier.
- The background is divided into coloured fields to simplify localization of the laboratory results.

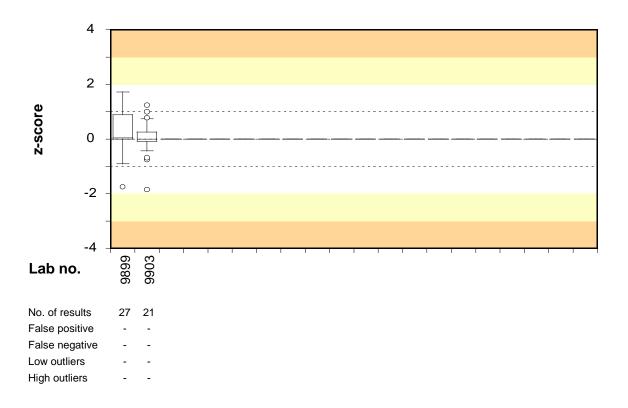
^{* &}lt; [smallest value of the box - $1.5 \times$ (largest value of the box - smallest value of the box)] or > [largest value of the box + $1.5 \times$ (largest value of the box - smallest value of the box)]











Test material, quality controls and processing of data

Description of the test material

This round comprised three test items with different microorganism composition. The test material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd [2]. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition and approximate concentrations in the samples obtained at the 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 be used without any problem.

Sample ¹	Microorganisms	Strain co	llection no.	cfu/100 ml ²
		SLV (own)	Reference ³	
A	Escherichia coli	165	CCUG 43600	14
	Klebsiella pneumoniae	537	_	54
	Kluyveromyces marxianus	439	CBS G99-106	74
	Phialophora malorum	545	From water	130
	Staphylococcus cohnii	462	CCUG 35411	31 *
В	Enterobacter aerogenes	099	ATCC 13 048	1400
	Aeromonas hydrophila	081	CCUG 45103	2800
	Clostridium perfringens	442	CCUG 43593	25
	Issatchenkia orientalis	498	CCUG 35869	3
	Cladosporium cladosporoides	488	CBS 812.96	8
С	Escherichia coli	532	CCUG 48891	50
	Citrobacter freundii	091	CCUG 43597	44
	Streptomyces sp.	548	From water	52
	Clostridium bifermentans	009	CCUG 43592	1700
	Stenotrophomonas maltophilia	041	CCUG 46537	20*

Table 2 Microorganisms present in the samples

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

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

3 Origin or typing collection no.; ATCC: American Type Culture Collection; CCUG: Culture Collection University of Gothenburg, Sweden; CBS: Centraalbureau vor Schimmelcultures, Utrecht, Holland; – or "From water" indicate a strain from 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 2 to 3 % of the number of vials produced from the mixtures. The largest differences between vials were 5, 10 and 4 mg in mixture A, B and C respectively. The largest accepted difference is 15 mg (3 %).

Analysis parameter				Sa	mpl	e			
Method standard for analysis		\mathbf{A}^{1}			${\bf B}^{\;1}$			\mathbf{C}^{2}	
	cfu	I ₂	Т	cfu	I ₂	Т	cfu	I ₂	Т
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	68	0.6	1.2	43 ^b	1.0	1.4	47°	0.4	1.2
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	62	1.6	1.4	0	_	_	40	0.4	1.2
Escherichia coli (MF) m-Endo Agar LES according to SS 028167	14	0.6	1.7	0	_	_	25°	0.3	1.2
Presumptive Clostridium perfringens (MF) TSC Agar according to SS-EN ISO 14189:2016	_	_	_	25	0.3	1.2	17 ^b	0.3	1.3
Moulds (MF) Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192	13 ^a	0.6	1.5	8	1.3	2.2	_	_	_
Yeasts (MF) Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192	7 ^a		2.2	3	0.4	2.0	_	_	_
Actinomycetes (MF) Actinomycete Isolation Agar with cycloheximide according to SS 028212	_	_	_	_	_	_	26°	0.9	1.4
Culturable microorg., 3d 22 °C (pour plate) Yeast extract Agar according to SS-EN ISO 6222:1999	32	2.3	1.8	45	0.3	1.2	21	0.8	1.5

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

1 10 vials (sample C: 5 vials) analysed in duplicate, normally100 ml for MF and 1 ml for pour plate, analysed 18, 7 and 13 weeks ahead of the testing round for the sample A, B and C, respectively

a Determined for the volume 10 ml

b Determined for the volume 1 ml

c Determined for the volume 50 ml

- 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 deviating results. 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 <u>https://www2.slv.se/absint</u>.

References

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- 2. Peterz, M., Steneryd, A.-C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. J. Appl. Bacteriol. 74:143-148.
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Annex A Results of the participants, $cfu/100 \ ml$ (see also the note [#]). Susp. = suspected on membrane filter before confirmation. Results given as <1, <2, <10 and <100 are treated as zero. The fields with other results given as < 'value' and results given as > 'value' are **yellow**, and those results are not included in calculations or evaluations. This is also valid for results in **shaded columns**. A **hyphen** indicate that no result has been reported. **Figures written in bold in yellow fields** indicate outliers, false positive and false negative results. **Underlined zero values** indicate results characterized as 'False negative ?'. **Crossed out sample numbers** in a row indicate that the samples probably are mixed up. False positive and false negative

Lab no.	Sa	mple	-	cted coli		Coliform	bacter	ia (MF)		nermoto		E. (coli (MF)			orm bact		E. coli	("rapid"	MPN)
	•	вс	bac A	teria (MI B	F) C	•	в	с	colifor A	m bact. B	(MF) C	•	в	С	("ra A	pid" MP B	N) C	•	в	С
1131	_	23	- A	<u>в</u> -	<u>.</u>	A -	<u>в</u> -	ر	- A	<u>-</u>		A _	<u>ь</u> -	۰ -	A 56	84	58	A 10	<1	42
1237	3	2 1	-	-	-	47	1300	1700	-	-	-	12	<1	40	63	920	79	12	<1	48
1545		13	54	3300	69	54	1600	69 70	54	1600	32	13	0	32	44	1330	91	11	0	47
1594 1611		32 12	74	- 1020	60	54 74	1100 1020	78 60	- 60	- 0	16	11 9	0 0	39 34	46 77	1200 1203	90 50	10 10	0 0	46 29
1753		3 2	66	1210	84	66	1210	84	-	-	-	16	0	43	59	1350	91	12	0	54
1868		3 1	125	2500	64	57	1000	64	-	-	-	16	0	43	66	1295	85	19	0	49
1970 2050		13 31	51	2700	78	51 56	1100 1073	78 42	51	1200	78	0 5	0 0	43 42	- 59	- 1429	- 62	- 11	-0	- 42
2317		2 1	-	-	-	42	850	54	-	-	-	17	0	33	-	-	-	-	-	-
2386		32	129	2500	77	67	2500	77	-	-	-	12	0	51	78	1650	70	12	<1	38
2637 2704		23 31	-	-	-	- 44	- 1100	- 63	-	-	-	- 16	- 0	- 32	66 59	1445 1652	48 130	11 14	0	32 78
2745		23	57	910	60	57	910	60	57	0	32	17	0	32	-	-	-	-	-	-
2944		1 3	-	-	-	-	-	-	-	-	-	-	-	-	88.5	1013	59.1	19.2	<1	38.4
3055 3076		2 1 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076		23	-	-	-	-	-	-	-	-	-	-	-	-	80	1300	- 91	15	- 0	38
3155	3	2 1	-	-	-	56	959	65	55	<1	30	15	<1	35	64	1311	52	11	<1	30
3159		21	107	2900	64	49	1200	64	-	-	-	11	<1	30	56	1652	69.7	13.7	<1	50.4
3162 3164		23 13	-		-	- 57	- 1200	- 84	-		-	57	- 0	- 15	65 84	1553 1120	79 62	18 19	0 0	53 40
3305	2	1 3	-	-	-	62	1600	65	-	-	-	9	<1	34	64	1600	63	16	<1	41
3730		1 2	70	1900	100	-	-	-	62	0	26	-	-	-	-	-	-	-	-	-
3883 4015		13 32	71	2040	74	71	1230	74			-	28	<1 -	35	40 81	1290 1100	65 77	16 17	<1 <1	44 40
4288		3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4339		2 1	52	3900	84	52	1300	45	54	0	21	12	0	45	44	1300	57	9	0	29
4343 4356		32 13	- 48	- 2000	- 68	- 48	- 1400	- 68	- 48	- 0	- 31	- 8	- 0	- 51	62 48	1986 1300	68 74	19 12	0 0	38 35
4633		3 2	40	2000 -	- 00	40	1600	59	40	-	-	20	0	35	40	1100	66	12	0	38
4723	2	1 3	-	-	-	-	-	-	-	-	-	-	-	-	57	1300	77	11	0	42
4889		23	-	-	-	52	2200	72	-	-	-	15	0	38	46	1400	72	16	0	40
4980 5018		23 31	- 54	- 1750	- 57	- 54	- 880	- 57	-	-	-	- 16	- <1	- 29	76.4 68	1298 1000	73.8 73	9.3 16	<1 <1	42.9 39
5120		23	-	-	-	54	1600	71	55	0	34	11	0	49	56	1100	55	11	0	42
5128		1 2	-	-	-	-	-	-	48	<1	16	-	-	-	56	1010	62	12	<1	38
5201 5352		13 31	- 83	- 2650	- 77	38 83	2410 1550	30 77	- 18	- 0	-0	9 18	<1 0	27 18	-	-	-	-	-	-
5447		3 2	-	-	-	60	1100	110	-	-	-	15	0	46	-	-	-	-	-	-
5553		23	-	-	-	45	-	30	-	-	-	13	0	28	-	-	-	-	-	-
5858 5950	-	12 13	- 79	- 4200	- 71	49 79	485 1300	41 71	- 55	- 1000	- 30	11 12	0 <1	27 35	- 75	- 1553	- 61	- 17	- <1	- 40
6175		2 1	40	27	26	-	-	-	-	-	-	-	-	-	74	145	74	21	0	36
6182		31	53	1880	36	67	1880	78	-	-	-	14	0	42	72	1203	71	12	0	44
6233		2 1	62	4400	66	62	1200	66	-	-	-	13	0	40	68	1380	57	15	0	40
6253 6448	-	12 23	- 59	- 1800	- 150	- 59	1800	150	-	-	-	- 14	0	- 75	64	1500	66 -	14	<1	40
6456	3		-	-	-	80	800	58	-	-	-	49	<1	35	59	1400	74	14	<1	59
6563 6696	-	12	50	111	91	50	44	91	50	111	91	<1	<1	55	72	80	62	15	<1	28
6686 6801		32 21	-		-	-	-	-	-		-	-	-	-	59.1 -	1013	56 -	15	<1	42.9
7248		32	44	1500	57	44	<1	57	12	<1	27	18	<1	46	37	1300	59	13	<1	34
7442		1 2	148	3125	74	74	1450	74	-	-	-	17	<1	39	78	995	78	19	<1	41
7688 7728		23 13	62	1350	52	62 50	1350 1200	52 44	-		-	16 10	0	36 22	61	980	50	11	0	38
7876		3 1	68	3000	89	68	1200	89	58	950	18	16	<1	47	64	1014	93	18	<1	53
7930	3	2 1	68	1700	68	68	1100	68	-	-	-	14	<1	39	70	1100	78	14	<1	41
7946 7962		31 23	56 55	800 1100	51 65	52 55	780 1100	51 65	59 62	714 0	21 20	0 11	0 0	30 34	56 77	42	16	0 13	0	15 38
7962 7968		23	55 42	1100 2250	65 83	55 42	1100 1200	65 83	62 38	0	20 38	11 7	0	34 36	47	1414 2020	61 95	13 10	0	38 39
8019	2	13	58	3500	76	58	1200	76	49	0	20	13	0	54	57	1246	78	20	0	39
8068 8252			-	-	-	62	1800	42	48	0	19	0	0	42	62	1500	60	11	0	44
8252 8260	1 2	32 13	-		-	- 61	- 1107	- 63	-	-	-	- 16	- <1	- 35	74	1300	66 -	10	<1	45
8329		1 3	-	-	-	-	-	-	-	-	-	-	-	-	64	1526	54	17	<1	31
8380		1 2	52	2200	70	52	800	70	-	-	-	16	0	36	43	1034	60	8	0	30
8435 8569		31 32	- 65	- 2600	- 74	58 65	750 1300	65 74	47	0	9	15 16	0 0	44 43	- 73	- 1046	- 93	- 13	- 0	- 38
8626		23	43	1000	66	43	1000	66	43	1000	- 66	0	0	43 33		-		-	-	-
8628		1 3	-	-	-	30	600	1400	0	0	0	8	0	24	-	-	-	-	-	-
Mean						56	1231	65 12				13	0	37	62 10	1276	69	14	0	41
CV (%)						10	16	12				15	-	14	10	10	12	13	-	10

values are excluded, as well as other outliers, in the summarizing calculated results at the end of the table. The mean value (Mean) is the square of the mean value for the square root transformed results (mv). The coefficient of variation (CV) is the standard deviation (s) in percentage of the mean value for the square root transformed results. As means to calculate the z-values of your own, the appropriate values of mv and s are given at the end of the table. The x-values are obtained as the square roots of 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.

Lab no	ount	plate co	Total	(MF)	nycetes	Actino	;)	asts (MF	Yea	-)	ulds (MF	Мо	n	stridiun	Clo	e C.	umptive	Pres
		°C, 3 da		```				•			•		MF)	ngens (perfri		ingens	
	C	В	A	С	В	Α	С	В	Α	С	В	Α	С	В	Α	С	B	Α
113 123	23 27	0 28	32 37	-	-	-	۔ 10>	- <10	<10	- <10	- <10	<10	- <10	- 30	- 7	0	43	0
123	27	28 33	37 44	- 74	0	0	<10 0	<10	140	<10 0	<10	120	<10 0	30 42	0	- 1800	- 42	0
159	16	31	47	99	0	0	0	18	81	0	5	62	-	-	-	-	-	-
161	840	22	53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
175	24	37	33	58	0	0	0	0	91	0	0	67	210	28	0	210	28	0
186	20	30	37	-	-	-	-	-	-	-	-	-	-	-	-	167	9	0
197 205	15 21	30 38	30 41	- 66	-0	0	0 0	4 6	91 114	0 0	8 4	170 84	0	24	0	970 1873	24 41	0 0
203	26	35	38	-	-	-	-	-	-	-	-	-	-	11	0	- 1075	-	-
238	26	34	59	-	-	-	-	-	-	-	-	-	0	12	0	630	12	0
263	16	21	52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
270	19	34	54	-	-	-	-	-	-	-	-	-	0	33	0	930	33	0
274 294	9 16	23 28	45 55	-	-	-	-	-	-	-	-	-	-	- 39	- <1	-	39	-
305	13	33	29	-	-	-	-	-	-	-	-	-	<1	- 39	-	<1	- 39	<1
307	18	32	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
314	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
315	17	27	54	-	-	-	-	-	-	-	-	-	<1	44	<1	1250	44	<1
315	24	31	39	-	-	-	-	-	-	-	-	-	<1	16	<1	510	16	0
316 316	26 22	28 34	52 40	63 88	0 900	0 42	0 63	4 0	80 85	0 73	10 0	80 74	-	-	-	290	35	0
310	22	34 22	40 18	64	900 <1	42 <1	<1	7	80 80	<1	8	130	- <1	38	- <1	- 610	- 38	- <1
373	19	32	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
388	15	25	48	62	<1	<1	<1	2	84	<1	6	79	-	-	-	3200	36	<1
401	16	38	40	66	<1	<1	<1	4	90	<1	9	101	-	-	-	1550	36	<1
428	25 24	35	75	-	-	-	-0	-	-	-	-	- 80	-	-	- 0	-	-	-
433 434	24 15	24 30	49 43	40	0	0	0	3 5	120 73	0 0	8 5	127	0	23	-	2600 175	23 29	0 0
435	17	18	58	-	-	-	-	-	-	-	-	-	0	18	0	1300	18	0
463	50	25	43	50	0	0	0	7	80	0	6	68	-	-	-	-	-	-
472	25	27	61	47	0	0	0	5	77	0	4	86	-	-	-	2545	30	0
488	28	23	39	-	-	-	-	-	-	-	-	-	0	30	0	-	-	-
498 501	20 24	16 20	48 50	- 69	- <1	- <1	- <1	- 6	- 91	- <1	- 9	- 94	750 <1	25 32	<1 <1	750 2100	25 32	0 <1
512	24	30	49	60	0	0	0	81	61	0	11	72	0	28	0	520	28	0
512	17	17	52	-	-	-	-	-	-	-	-	-	<1	19	<1	27	19	<1
520	18	34	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
535	13	30	47	56	0	0	0	0	70	0	70	230	-	-	-	208	31	0
544 555	19	41	37	40	0	0	0	3	145	0	9	145	0 0	30 27	0	1900	30	0
585	- 18	- 14	- 52	-	-	-		-	-	-	-	-	0	27	0 0	- 3500	29	0
595	21	32	32	67	<1	<1	<1	3	92	<1	7	136	490	25	<1	-	-	-
617	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
618	21	21	66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
623	15	16	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
625 644	23 36	26 20	32 53		-	-	0	- 0	- 80	-0	0	170	-	-	-	-	-	-
645	13	18	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
656	25	<1	57	59	<1	<1	<1	9	145	<1	5	100	-	-	-	1609	31	<1
668	21	23	12	-	-	-	-	-	-	-	-	-	-	-	-	<1	23	<1
680	-	-	-	-	-	-	-	-	- 40	-	-	-	-	-	-	-	-	-
724 744	- 23	37 26	91 44	58	<1	<1	<1	5	19	<1	6	24	2300	27	<1	2300	27	<1
768	20	15	44	98	0	0	0	3	86	0	7	130	0	42	0	-	-	-
772	18	20	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
787	19	29	52	80	<1	<1	<1	6	120	<1	11	130	-	-	-	2700	41	<1
793	22	31	41	-	-	-	<1	<1	110	<1	12	110	<1	25	<1	890	25	<1
794 796	100 22	250 28	20 48	-	-		-	- 4	- 120	-0	- 5	- 50	2	10	0	1	10	0
796	22	32	40 51	-	-	-	0	4	77	0	11	75	0	23	0	- 1060	- 23	0
801	23	37	54	-	-	-	0	0	106	0	12	80	0	29	0 0	1400	29	Ő
806	15	28	32	-	-	-	0	4	95	0	9	100	0	27	0	-	-	-
825	22	21	34	-	-	-	-	-	-	-	-	-	<1	45	<1	-	-	-
826 832	55 26	30 23	52 37	- 52	- <1	- <1	- 1	- 3	- 71	- <1	- 2	- 60	-	-	_	1883 1010	37 51	<1 <1
838	20 19	23 25	37 28	52	-	-		-	-	<1	-	- 00	-	-		- 1010	-	-
843	20	25	44	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
856	18	49	61	-	-	-	-	-	-	-	-	-	230	33	0	230	33	0
862	15	5	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
862 Mea	27 20	11 27	31 43	- 63	- 0	- 0	0	5 3	65 90	0	5 6	47 94	0	34 28	0	- 1204	- 29	- 0
CV (%	20 11	14	43 12	11	-	-	-	3 65	90 12	-	o 41	94 21	-	20 17	-	45	29 17	-
2. (/																		

Lab no.	Sai	mple		cted col cteria (M		Coliforn	n bacter	ia (MF)		hermoto m bact.		E.	coli (M	F)		orm bac apid" MF		E. coli	("rapid'	MPN)
	А	вс	A	B	., с	А	в	С	A	B	<u>c</u>	Α	в	С	A .	В	<u>c</u>	А	в	С
8663	1	32	51	2700	78	51	1100	78	48	0	37	15	0	62	78	1200	62	24	0	39
8742		1 2	-	-	-	55	1300	43	-	-	-	6	<1	28	-	-	-	-	-	-
8751		23	-	-	-	-	-	-	-	-	-	-	-	-	70	740	78	15	0	59
8766		32	50	1700	50	50	1000	50	40	400	2	13	0	19	66	1046	31	12	0	19
8862 8898		31 13	93 57	2800 3270	61 91	37 57	1100 2360	61 91	-	-	-	8 9	0 0	32 45	61 53	1203 1541	62 85	17 14	0 0	44 64
9436		2 1	57	3100	60	57	1100	91 60	57	0	- 18	9 10	0	43 37	63	1044	108	14	0	64 57
9524		3 1		-	- 00	55	980	57		-		10	<1	39	66	1467	56	19	<1	32
9736		32	-	-	-	-	-	-	-	-	-	-	-	-	73	1120	49	22	0	32
9899	2	31	65	1268	87	65	1268	87	-	-	-	17	0	55	59	1287	93	11	0	47
9903	2	31	48	2725	82	48	1292	82	81	1200	20	14	0	20	-	-	-	-	-	-
n			39	39	39	60	59	60	27	27	27	60	60	60	61	61	61	61	61	61
Min Max			40 148	27 4400	26 150	30 83	0 2500	30 1700	0 81	0 1600	0 91	0 57	0 0	15 75	37 88.5	42 2020	16 130	0 24	0 0	15 78
IVIAX			140	4400	150	03	2500	1700	01	1000	91	57	0	75	00.0	2020	130	24	0	10
Median			57	2200	70	55	1200	65	51	0	21	13	0	36	64	1298	67	14	0	40
Mean						56	1231	65				13	0	37	62	1276	69	14	0	41
CV (%)						10	16	12				15	-	14	10	10	12	13	-	10
False po	- 141					0	0	0				0	0	0	0	0	0	0	0	0
False po						0	1	0				5	0	0	0	0	0	1	0	0
Outliers						0	1	0				0	0	0	0	4	1	Ó	Ő	1
Outliers						0	0	3				2	0	0	0	0	0	0	0	1
		-																		
Low lim			40	27	26	30	485	30	0	0	0	5	0	15	37	740	31	8	0	19
High lim	it O	ĸ	148	4400	150	83	2500	110	81	1600	91	28	0	75	89	2020	130	24	0	64
						7 455	05 000	0.054				0.004	0.000	0.000	7 000	05 740	0.004	0 740	0.000	0.074
mv (√Mean	,					7.455	35.080	8.054				3.601	0.000	6.083	7.902	35.718	8.331	3.742	0.000	6.371
s	/					0.709	5.589	0.995				0.551	0.000	0.872	0.760	3.430	0.965	0.471	0.000	0.650
(CV*mv/	100)				0.1.00	0.000	0.000				0.001	0.000	0.072	0.100	0.100	0.000	0.111	0.000	0.000
u _{rel,mv} (9 (100*s/ 1		v)				1.2	2.1	1.6				2.1		1.9	1.2	1.3	1.5	1.6		1.3
x (√Resul	t)																			
z ([x-mv]/s)																			

cfu/ml

	umptive ingens			ostridiu ingens		Мо	ulds (M	F)	Ye	asts (M	F)	Actino	mycetes	s (MF)		l plate c		Lab no.
A	B	(IMF) C	A	B	(WIF) C	Α	в	С	Α	в	С	А	в	С	22±2	°C, 3 da B	iys C	-
		-			-	А		ι	A	Б	L	A	Б	ι			-	
0	31	1500	0	31	0	-	-	-	-	-	-	-	-	-	37	40	21	8663
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45	22	16	8742
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	56	24	20	8751
0	27	3600	-	-	-	120	10	0	64	1	0	0	0	50	45	19	23	8766
0	49	2800	0	49	0	63	11	0	67	0	0	0	0	59	36	14	21	8862
0	33	4360	-	-	-	108	8	0	100	5	0	0	0	58	42	26	22	8898
0	24	1900	-	-	-	41	6	0	79	0	0	0	0	62	52	34	17	9436
<1	30	<1	<1	30	<1	-	-	-	-	-	-	-	-	-	39	17	23	9524
0	28	0	-	-	-	136	13	0	72	4	0	0	0	64	39	26	22	9736
0	14	905	-	-	-	95	9	0	86	4	0	0	0	70	54	35	29	9899
0	28	1258	-	-	-	81	3	0	81	3	0	0	0	74	58	33	23	9903
			-						-									
47	47	47	36	36	35	39	39	39	39	39	39	29	29	29	78	78	77	n
0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	12	0	9	Min
0	51	4360	7	49	2300	230	70	73	145	81	63	42	900	99	91	250	840	Max
	~~ -	1050		~~~			_									~~~		
0	29.5	1258	0	29	0	90	7	0	85	4	0	0	0	62	44	28	21	Median
0	29	1204	0	28	0	94	6	0	90	3	0	0	0	63	43	27	20	Mean
-	17	45	-	17	-	21	41	-	12	65	-	-	-	11	12	14	11	CV (%)
0	0	0	1	0	6	0	0	1	0	0	2	1	1	0	0	0	0	False pos.
0	1	6	0	1	0	1	0	0	1	0	0	0	0	0	0	2	0	False neg.
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	Outliers <
0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	4	Outliers >
0	9	1	0	10	0	24	0	0	61	0	0	0	0	40	18	11	9	Low limit
0	51	4360	0	49	0	230	13	0	145	18	0	0	0	99	75	49	36	High limit
0.000	5.368	34.697	0.000	5.290	0.000	9.697	2.410	0.000	9.479	1.602	0.000	0.000	0.000	7.947	6.595	5.179	4.513	mv
0.000	0.926	15.542	0.000	0.918	0.000	2.025	0.990	0.000	1.115	1.046	0.000	0.000	0.000	0.875	0.823	0.723	0.504	s
	2.5	7.0		2.9		3.4	6.7		1.9	10.6				2.0	1.4	1.6	1.3	u _{rel,mv} (%)
																		x
																		z

l eh ne	Comula	Successful coliform	Calif	orm boo	torio	Sucn	thormoto	lorant	E	ooli (M	E)	Calif	orm bac	torio	E coli	("rapid	
Lab no.	Sample	Suspected coliform bacteria (MF)	Colli	orm bac (MF)	teria		thermoto orm bact.		<i>E.</i>	coli (M	F)		apid" MF		E. coli	(rapid	WPN)
	АВС	А В С	Α	Ъ	С	Α	В	`c´	Α	В	С	À	В	ć	Α	В	С
1131	1 2 3												-4.000		-1.231	0.000	0.168
1237	321			0.175					-0.248	0.000*			-1.571	0.577	-0.590	0.000	0.857
1545 1594	2 1 3 1 3 2			0.880 -0.342	0.254				0.009 -0.516		-0.488 0.186	-1.670	0.219 -0.314	1.251 1.197	-0.903 -1.231	0.000 0.000	0.745 0.633
1611	3 1 2			-0.562					-1.090		-0.289		-0.302		-1.231		-1.518
1753	1 3 2			-0.053	1.116				0.724		0.544	-0.291	0.299	1.251	-0.590	0.000	1.504
1868	231			-0.619					0.724	0.000		0.292	0.078	0.920	1.310	0.000	0.968
1970	2 1 3			-0.342					0.475		0.544	0.001	0.000	0 474	0.000	0.000	0.400
2050 2317	231 321			-0.416 -1.060					-2.475 0.947	0.000	-0.388	-0.291	0.608	-0.474	-0.903	0.000	0.168
2386	1 3 2			2.669					-0.248		1.213	1.223	1.429	0.037	-0.590	0.000	-0.319
2637	1 2 3											0.292	0.669	-1.453	-0.903		-1.100
2704	231			-0.342					0.724		-0.488	-0.291	1.436	3.181	-0.001	0.000	3.787
2745 2944	123 213		0.134	-0.879	-0.310				0.947	0.000	-0.488	1 000	-1.134	0.667	1 250	0.000	0.260
2944 3055	3 2 1											1.960	-1.134	-0.007	1.559	0.000	-0.209
3076	2 3 1																
3145	1 2 3											1.371				0.000	
3155	321			-0.736						0.000		0.128		-1.160	-0.903		-1.376
3159 3162	321 123		-0.641	-0.079	-0.054				-0.516	0.000	-0.694	-0.551 0.210		0.018	-0.086 1.063		1.120 1.399
3164	2 1 3		0 134	-0.079	1 1 1 6				4.000	0.000	-2.533		-0.657		1.310		-0.072
3305	2 1 3			0.880					-1.090		-0.289	0.128	1.248		0.548		0.049
3730	3 1 2														Ι.		
3883	2 1 3		1.370	-0.002	0.551				3.067	0.000	-0.191		0.058		0.548		0.403
4015 4288	1 3 2 1 3 2											1.444	-0.744	0.460	0.809	0.000	-0.072
4200	321		-0.343	0.175	-1.352				-0.248	0.000	0.717	-1.670	0.098	-0.809	-1.575	0.000	-1.518
4343	1 3 2											-0.037		-0.088		0.000	
4356	2 1 3			0.418					-1.401		1.213	-1.282		0.281	-0.590		-0.701
4633	132 213		-0.641	0.880	-0.375				1.580	0.000	-0.191		-0.744 0.098		-0.590		-0.319
4723 4889	123		-0 343	2.116	0 433				0.494	0 000	0.093	-0.464 -1.474		0.460 0.160	-0.903 0.548		0.168 -0.072
4980	1 2 3		0.040	2.110	0.400				0.404	0.000	0.000	1.103		0.269	-1.470		0.275
5018	231			-0.969						0.000			-1.194	0.220	0.548		-0.195
5120	123		-0.150	0.880	0.374				-0.516	0.000	1.051		-0.744		-0.903		0.168
5128 5201	3 1 2 2 1 3		-1.910	2.507	-2 580				-1.090	0.000	-1.016	-0.551	-1.148	-0.474	-0.590	0.000	-0.319
5352	2 3 1			0.768	0.724				1.164		-2.109						
5447	1 3 2			-0.342					0.494		0.802						
5553	123		-1.052		-2.589				0.009	0.000							
5858	3 1 2			-2.336					-0.516		-1.016	0.007	4 070	0 5 40	0.000	0.000	0.070
5950 6175	2 1 3 3 2 1		2.021	0.175	0.374				-0.248	0.000	-0.191		1.076		1.785	0.000	-0.072
6182	231		1.030	1.481	0.781				0.255	0.000	0.456		-0.302		-0.590		0.403
6233	321			-0.079					0.009	0.000			0.417		0.278		-0.072
6253	3 1 2				1 0 0 0							0.128	0.878	-0.215	-0.001	0.000	-0.072
6448 6456	123 321			1.314					0.255		2.954	-0.291	0.495	0.201	-0.001	0.000	2.016
6563	3 1 2			-1.216					4.000		1.528		-4.000		0.278		-1.662
6686	1 3 2		0.011							0.000			-1.134		0.278		0.275
6801	321																
7248	1 3 2		-1.158		-0.507				1.164		0.802		0.098		-0.290		-0.832
7442 7688	3 1 2 1 2 3			0.537 0.297						0.000 0.000			-1.217 -1.287		1.310 -0.903		0.049
7728	2 1 3			-0.079						0.000		0.121	1.207	1.000	0.000	0.000	0.013
7876	231		1.116	-0.079	1.387				0.724	0.000	0.886				1.063		
7930	321			-0.342					0.255	0.000			-0.744		-0.001		
7946 7962	231 123			-1.280 -0.342					-0.516	0.000 0.000	-0.694	-0.551 1.148	-4.000	- 4.000 -0.540	-0.290		-3.846
7968	1 2 3			-0.079						0.000		-1.377		1.466	-0.230		-0.195
8019	2 1 3			-0.079						0.000			-0.122		1.550		-0.195
8068	231		0.591	1.314	-1.581					0.000	0.456		0.878		-0.903		0.403
8252	1 3 2 2 1 3		0.504	0.004	0 4 4 7				0.704	0.000	0.404	0.921	0.098	-0.215	-1.231	0.000	0.518
8260 8329	213		0.501	-0.324	-0.117				0.724	0.000	-0.191	0 128	0.976	-1 018	0 800	0.000	-1 237
8380	3 1 2		-0.343	-1.216	0.314				0.724	0.000	-0.095		-1.039		-1.939		-1.376
8435	2 3 1			-1.377						0.000							
8569	1 3 2			0.175					0.724	0.000		0.844	-0.984	1.359	-0.290	0.000	-0.319
8626	123			-0.619					1 404		-0.388						
8628 8663	2 1 3 1 3 2			-1.894 -0.342						0.000 0.000		1 222	-0.314	-0 474	2.457	0.000	-0 195
8742	3 1 2			0.175						0.000			0.014	0.7/7		0.000	0.100
8751	1 2 3											0.611	-2.483	0.518	0.278	0.000	
8766	1 3 2			-0.619					0.009		-1.976		-0.984		-0.590	0.000	-3.098
8862	231			-0.342						0.000			-0.302		0.809		0.403
8898	2 1 3			2.415						0.000			1.031		-0.001		2.507
9436 9524	321 231			-0.342 -0.675						0.000 0.000			-0.993 0.753		0.278	0.000	1.814
9736	1 3 2		0.004	0.070	0.007				0.700	0.000	0.100		-0.657			0.000	
9899	2 3 1		0.857	0.095	1.279				0.947	0.000	1.528		0.046		-0.903		
9903	2 3 1			0.155						0.000							

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

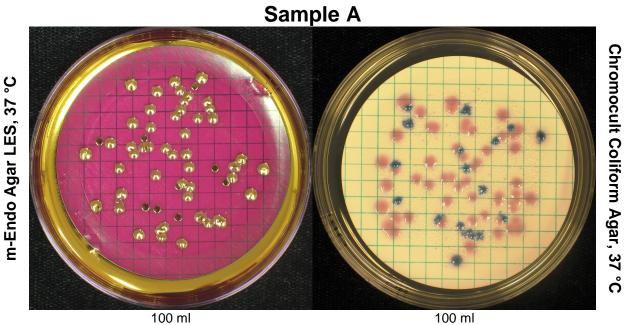
positive results no z-scores can be calculated. Z-scores form outliers are not real z-
scores but a practical means to express also the results from the outliers. Very low and
high values are here limited to -4 and $+4$, respectively.

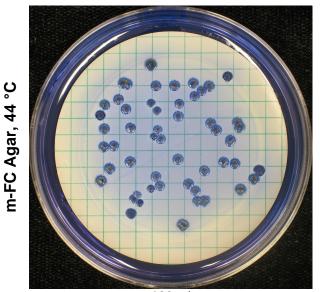
	esump				ostridiu		Мс	oulds (M	IF)	Ye	easts (M	IF)	Actino	mycete	s (MF)		l plate c		Lab no.
A	rfringe B		C C	A	ringens B	(mr) C	Α	в	С	Α	в	С	Α	в	С	A 22	°C, 3 da B	C C	
0.00			-									-			-	-1.140		0.561	1131
					0.204	0.000		-2.435			-1.531	0.000				-0.622		1.356	1237
0.00	0 1.2	02	0.497	0.000	1.298	0.000		-0.685 -0.176	0.000 0.000	2.111 -0.429	-0.575 2.524	0.000 0.000	0.000	0.000	0.749 2.289	0.047 0.317	0.782	1.163 -1.017	1545 1594
							-0.900	-0.170	0.000	-0.429	2.524	0.000	0.000	0.000	2.205		-0.676	4.000	1611
	0 -0.0			0.000	0.002		-0.747	-2.435	0.000	0.055	-1.531	0.000	0.000	0.000	-0.379	-1.033	1.250	0.766	1753
	0 -2.5			0.000	0.400	0.000	1.050	0 400	0.000	0.055	0.004	0.000				-0.622		-0.081	1868
	0 -0.5 0 1.1			0.000	-0.426	0.000		0.423 -0.414			0.381 0.810	0.000 0.000	0.000	0.000	0 202	-1.358 -0.233	1.363	-1.269 0.138	1970 2050
0.00	•		0.002	0.000	-2.150		0.200	0	0.000		0.010	0.000	0.000	0.000	0.202	-0.523	1.019	1.163	2317
0.00	0 <mark>-2.0</mark>	57	-0.618	0.000	-1.990	0.000										1.321	0.902	1.163	2386
0.00	0 0.4	07	0.270	0.000	0.496	0.000											-0.825 0.902		2637 2704
0.00	0 0.4	07	-0.270	0.000	0.490	0.000											-0.530		2704
0.00	0 0.9	48		0.000	1.041	0.000										0.999		-1.017	2944
																-1.470		-1.800	3055
																-0.425	0.661	-0.536	3076 3145
0.00	0 1.3	67	0.042	0.000	1.464	0.000										0.916	0.024	-0.773	3145
	0 -1.4			0.000	-1.406	0.000										-0.425	0.538	0.766	3159
0.00	0 0.5	92	-1.137						0.000		0.381	0.000	0.000	0.000		0.749	0.156	1.163	3162
0.00	0 0 8	61	-0.643	0 000	0.953	0 000	-0.541 0.842	-2.435 0.423	0 000	-0.232 -0.479	-1.531 0.998	0.000	0.000	0.000	1.639	-0.328	0.902	0.352	3164 3305
0.00	0.0	51	5.040	0.000	0.000	0.000	0.042	0.420	0.000	5.475	0.000	0.000	0.000	0.000	0.000	-0.825		-0.305	3730
0.00			1.407				-0.399	0.040	0.000		-0.179	0.000	0.000		-0.084	0.405	-0.248	-1.269	3883
0.00	0 0.6	83	0.301				0.174	0.596	0.000	0.007	0.381	0.000	0.000	0.000	0.202	-0.328		-1.017	4015
0.00	0 -0.6	18	1 0/18	0 000	-0.538	0.000	-0 372	0.423	0.000	1 32/	0.125	0.000					1.019 -0.387		4288 4339
	0.0			0.000	0.000	0.000		-0.176	0.000	-0.838	0.606	0.000	0.000	0.000	-1.855				4339
	0 -1.2			0.000	-1.141	0.000										1.241	-1.295		4356
0.00		4.0	1 0 1 0				-	0.040	0.000	-0.479	0.998	0.000	0.000	0.000			-0.248	4.000	4633
0.00	0 0.1	18	1.013	0 000	0.204	0.000	-0.209	-0.414	0.000	-0.631	0.606	0.000	0.000	0.000	-1.248		0.024 -0.530	0.966 1.545	4723 4889
0.00	0 -0.3	97	-0.470		-0.316	0.000											-1.631		4980
0.00	0 0.3	12	0.716	0.000	0.400	0.000		0.596			0.810			0.000		0.579	-0.978	0.766	5018
	0 -0.0				0.002	0.000	-0.598	0.916	0.000	-1.497	4.000	0.000	0.000	0.000	-0.230		0.413	1.163	5120
0.00	0 -1.0	90	-1.898	0.000	-1.014	0.000											-1.460 0.902		5128 5201
0.00	0 0.2	16	-1.305				2.701	4.000	0.000	-0.998	-1.531	0.000	0.000	0.000	-0.530		0.413		5352
0.00			0.572		0.204	0.000	1.158	0.596	0.000		0.125	0.000	0.000	0.000	-1.855	-0.622	1.693	-0.305	5447
0.00		10	4 574		-0.102	0.000										0 740	4 000	0 500	5553
0.00	0 0.0	19	1.574		0.104 -0.316	0.000	0.970	0.238	0.000	0 101	0.125	0.000	0.000	0.000	0 272	-1.140	-1.988 0.661		5858 5950
				0.000	0.010		0.070	0.200	0.000	0.101	0.120	0.000	0.000	0.000	0.272	1.140	0.001	0.100	6175
																	-0.825		6182
																	-1.631		6233
							1 650	-2.435	0 000	-0 479	-1.531	0.000					-0.111 -0.978	0.561 2.950	6253 6448
									0.000	00		0.000					-1.295		6456
	0 0.2		0.348				0.150	-0.176	0.000	2.299	1.336	0.000	0.000	0.000	-0.304	1.161		0.966	6563
0.00	0 -0.6	18														-3.806	-0.530	0.138	6686
0.00	0 -0.1	86	0.853	0 000	-0.102		-2.369	0.040	0.000	-4.000	0.606	0.000	0.000	0.000	-0.379	3.580	1.250		6801 7248
0.00	0 0		0.000	0.000	002		2.000	0.0.0	0.000		0.000	0.000	0.000	0.000	0.010		-0.111	0.561	7442
1				0.000	1.298	0.000	0.842	0.238	0.000	-0.184	0.125	0.000	0.000	0.000	2.232	0.047	-1.806	-0.081	7688
0.00	0 1 4	10	1 1 1 1				0.940	0.016	0.000	1 224	0.910	0.000	0.000	0.000	1.140		-0.978		7728 7876
	0 1.1 0 -0.3			0.000	-0.316	0.000		0.916 1.065			0.810 -1.531	0.000	0.000	0.000	1.140		0.285 0.538		7876
	0 -2.3				-2.318	2.300	2.001					2.300					4.000		7946
0.07	o o -	4.0	0.400	0.000	0 500	0.000		-0.176		-	0.381						0.156		7962
	0 -0.6 0 0.0				-0.538 0.104			0.916 1.065			0.381 -1.531						0.661 1.250		7968 8019
0.00	0.0	13	5.175		-0.104			0.596			0.381						0.156		8068
1					1.546											-0.928	-0.825	0.352	8252
	0 0.7						0.007	4 000	0.000	0.044	0.405		0.000	0.000	0.044		0.413		8260
0.00	0 1.9	16	-0.188				-0.964	-1.006	0.000	-0.944	U.125		0.000	0.000	-0.841		-0.530 -0.248		8329 8380
0.00	0			0.000		0.000											-0.248		8435
	0 0.4	07	-1.257		0.496											1.478	2.519	-0.536	8569
1				0.000	0 500	0.000	4 400	0.470	0.000	4	0.000	0.000					-4.000		8626
0.00	0 0.2	16	0 260		0.590 0.303		-1.403	-0.176	0.000	-1.271	0.606	0.000					-2.576 1.584		8628 8663
0.00	J 0.2	10	5.200	0.000	0.000	0.000											-0.676		8742
1																1.080	-0.387	-0.081	8751
	0 -0.1			0 0	4.05.1	0.00-				-1.326				0.000			-1.134		8766
	0 1.7 0 0.4			0.000	1.864	0.000		0.916 0.423			-1.531 0.606			0.000 0.000			-1.988 -0.111		8862 8898
	0 -0.5							0.423			-1.531			0.000			0.902		9436
0.00	0 0.1	18		0.000	0.204	0.000										-0.425	-1.460	0.561	9524
	0 -0.0		0 207					1.208			0.381			0.000			-0.111		9736
	0 -1.7 0 -0.0							0.596 -0.685			0.381 0.125			0.000 0.000			1.019 0.782		9899 9903
0.00	- 0.0		5.500				0.044	0.000	0.000	5.420	0.120	0.000	0.000	0.000	5.7 40		5.7 62	0.001	

Lab no.	Sample	e	Suspect bact	ted co eria (l		Colif	orm bac (MF)	teria	Susp. th colifor	nermoto m bact.		E.	coli (M	F)	Coliform bacteria ("rapid" MPN)			E. coli	E. coli ("rapid" MPN	
	ABC	;	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С
n			0	0	0	60	58	60	0	0	0	55	60	60	61	61	61	60	61	61
Min						-2.788	-4.000	-2.589				-2.475	0.000	-2.533	-2.394	-4.000	-4.000	-1.939	0.000	-3.846
Мах						2.334	2.669	4.000				4.000	0.000	2.954	1.980	2.690	3.181	2.457	0.000	3.787
Median							-0.079	0.070				0.255			0.128			-0.001	0.000	
Mean SD						0.000 1.000	-0.069 1.122	0.200 1.312				0.145 1.239	0.000 0.000	0.000 1.000	0.000 1.000	-0.262 1.389	-0.066 1.116	0.000 1.000	0.000 0.000	-0.001 1.205
z<-3						0	1	0				0	0	0	0	4	1	0	0	2
-3≤z<-2						1	1	2				2	0	2	3	1	1	0	0	0
2 <z≤3< td=""><td></td><td></td><td></td><td></td><td></td><td>3</td><td>4</td><td>1</td><td></td><td></td><td></td><td>0</td><td>0</td><td>2</td><td>0</td><td>2</td><td>1</td><td>2</td><td>0</td><td>3</td></z≤3<>						3	4	1				0	0	2	0	2	1	2	0	3
z>3						0	0	3				3	0	0	0	0	1	0	0	1

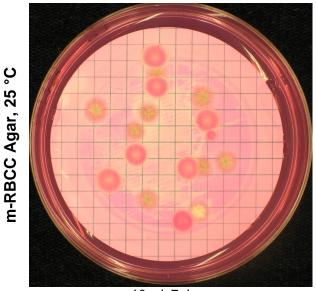
Lab no.		l plate c °C, 3 da		s (MF)	mycete	Actino	Yeasts (MF)			Moulds (MF)				sumptive C. Clostridium ringens (MF) perfringens (MF)				
	C	B	A	С	в	Α	С	В	Α	С	в	Α	<u>с</u>	B	A	C	B	A
n	77	76	78	29	28	28	37	39	38	38	39	38	29	35	35	41	46	47
Min	-3.001	-4.000	-3.806	-1.855	0.000	0.000	0.000	-1.531	-4.000	0.000	-2.435	-2.369	0.000	-2.318	0.000	-2.168	-2.558	0.000
Max	4.000	4.000	3.580	2.289	0.000	0.000	0.000	4.000	2.299	0.000	4.000	2.701	0.000	1.864	0.000	2.016	1.916	0.000
Median	0.138	0.156	0.047	-0.084	0.000	0.000	0.000	0.381	-0.257	0.000	0.238	-0.105	0.000	0.104	0.000	0.050	0.068	0.000
Mean	0.208	0.000	-0.003	0.000	0.000	0.000	0.000	0.103	-0.105	0.000	0.103	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SD	1.321	1.183	1.153	1.000	0.000	0.000	0.000	1.176	1.181	0.000	1.176	1.000	0.000	1.000	0.000	1.000	1.000	0.000
Sum																		
12	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
28	0	1	3	0	0	0	0	0	0	0	4	1	0	2	0	1	3	0
29	1	1	1	2	0	0	0	1	3	0	0	1	0	0	0	1	0	0
16	4	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0

Annex C – photos

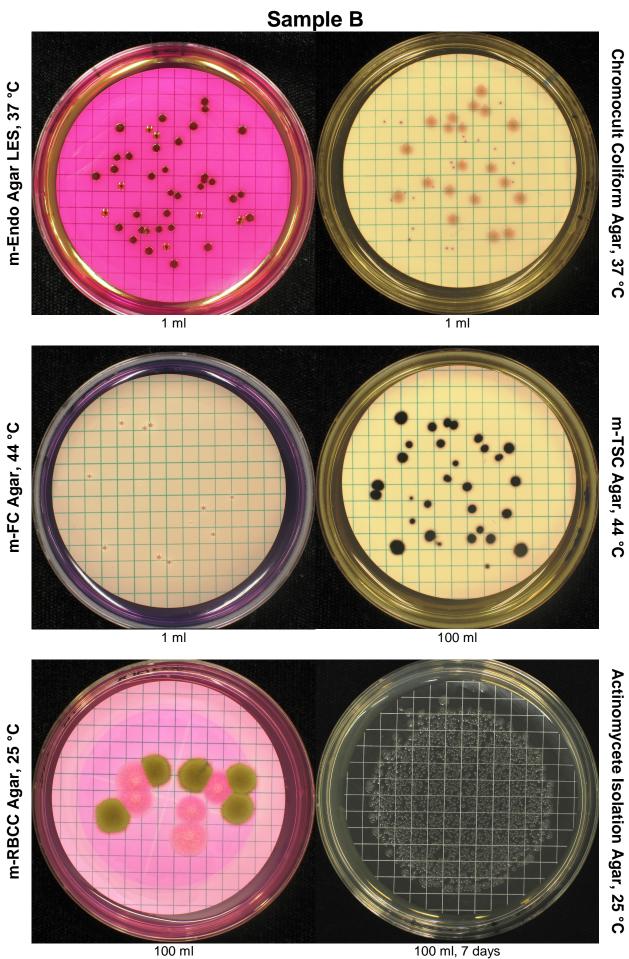




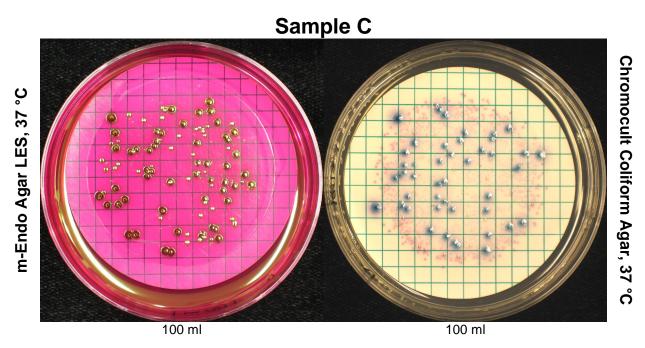
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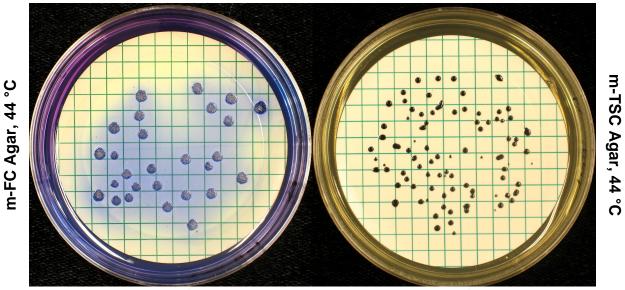


10 ml, 7 days



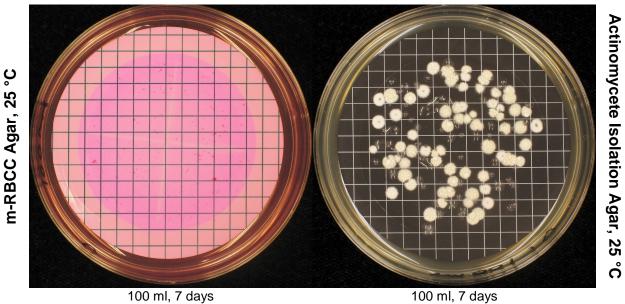
100 ml





100 ml

10 ml



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PT reports published 2018

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

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

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

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

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

PT reports published 2019

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

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

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

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

The National Food Agency's PT program offers

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

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

The National Food Agency's reference material

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

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