

Proficiency Testing

Drinking Water Microbiology

– March 2013

by Tommy Šlapokas and Kirsi Mykkänen



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) schemes.

In a proficiency test, identical test material is analysed by a number of laboratories using their routine methods. The laboratories report their results and 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 used by laboratories with respect to various types of organisms.
- Expert support
- Tool for inspections regarding accreditation.
- Free extra material for follow-up analyses

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

The National Food Agency's reference material

As a complement to the proficiency testing, National Food Agency also produces reference material (RM) for internal quality control: a total of 7 RM for food and drinking water microbiological analyses, including pathogens, are available.

Information available on our website: www.slv.se/RM

Edition

Version 1 (2013-06-17)

Editor in chief

Annika Rimland, Head of Science Department, National Food Agency

Responsible for the scheme

Tommy Šlapokas, Microbiologist, Microbiology Division, National Food Agency

PT March 2013 is registered as no. 659/2013 at the National Food Agency, Uppsala

Proficiency testing

Drinking Water Microbiology

March 2013



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

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

Clostridium perfringens with MF

Moulds with MF

Yeasts with MF

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

Tommy Šlapokas ¹
Kirsti Mykkänen ^{1,2}

¹ Compilation and report writing ² Laboratory work

National Food Agency, Microbiology Division, Box 622, SE-751 26 Uppsala, Sweden

Abbreviations and explanations

Common media in text and/or tables

LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (acc.to EN-ISO 9308-:2000)
m-FC	m-FC Agar (acc. 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 (acc. to SS 028192)
YeA	Yeast extract Agar (acc. to EN ISO 6222:1999)
CCA	Chromocult Coliform Agar® (Merck)
Colilert	Colilert® Quanti-Tray® (IDEXX Inc.)

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

Method tables for the analysis parameters

Tot n	total number of laboratories that reported methods and numerical results
n	number of results except false results and outliers
Mv	mean value (with deviating results excluded)
Med	median value (with deviating 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
	global results for the analysis
601	remarkably low result
278	remarkably high result or CV or many deviating results

Contents

Abbreviations and legends	2
General information on results evaluation	4
Results of the PT round March 2013	4
- General outcome	4
- Coliform bacteria (MF)	6
- Suspected thermo-tolerant coliform bacteria (MF).....	8
- <i>Escherichia coli</i> (MF)	9
- Coliform bacteria and <i>E. coli</i> (rapid method, MPN)	12
- <i>Clostridium perfringens</i> (MF)	14
- Moulds and yeasts (MF)	16
- Culturable microorganisms 22 °C, 3 days	18
Outcome of the results and laboratory assessment	20
- General information about reported results	20
- Assessment of the performance	20
- Z-scores, box plots and deviating results for each laboratory	20
Test material, quality control and processing of data	24
- Test material and its content	24
- Quality control of the mixtures	24
- Processing of numerical results	25
- Processing of methods data	26
References	27
Annex A – All reported results	28
Annex B – Z-scores of the results	32
Annex C – Photo example of colony appearance on some media	36

General information on results evaluation

The percentages of false results and outliers are compiled in **table 1**. These deviating results are excluded in most calculations. Descriptions of the histograms and calculation of outliers are mentioned briefly in the section "Evaluation of numerical results" at the end of this report and more detailed in the scheme protocol (1).

The proficiency testing program organised by the National Food Agency is accredited according to EN ISO/IEC 17043. Because it states that results might be grouped based on the method used, it is mandatory for participants to give such information. Results for various method groups are now accounted for per parameter.

The method information gathered is not always easy to interpret. Sometimes there is no consistency between the standard referred to and the information given regarding various method details. Results from laboratories with ambiguous details are either excluded from or placed in the group "Other/Unknown" in the tables, together with results from methods used only by some individual laboratories.

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in various tables together with the group means etc. The measure of dispersion is not shown for groups with less than 7 results, except when there are only two groups to compare results for.

Results of the PT round March 2013

General outcome

Test items were sent to 100 laboratories, 35 in Sweden, 52 in other Nordic countries and 13 in other countries. 95 laboratories reported results.

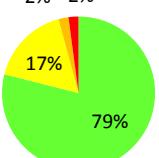
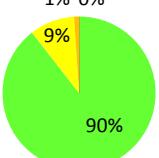
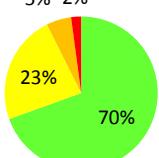
Microorganisms and parameters of analyses are given in **table 1**. For the MF analyses the parameters *suspected colonies of coliform bacteria* on primary media for quantification could be reported as well. The results from suspected colonies are only used as basis for interpretations and discussions.

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

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

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

Table 1 Microorganisms in each mixture and percentages of deviating results (F%: false positive or false negative, Out: outliers)

Mixture	A			B			C			
Percentage of laboratories with	 0 deviating results: 79%			 0 deviating results: 90%			 0 deviating results: 70%			
No. of evaluable results	524			523			522			
No. of deviating results *	26 (5 %)			11 (2 %)			39 (7 %)			
Microorganisms	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Phialophora malorum</i> <i>Kluyveromyces marxianus</i> <i>Staphylococcus xylosus</i>			<i>Citrobacter freundii</i> <i>Aeromonas hydrophila</i> <i>Clostridium perfringens</i>			<i>Escherichia coli</i> (gas neg.) <i>Cronobacter sakazakii</i> <i>Serratia marcescens</i> <i>Staphylococcus cohnii</i>			
Analysis	Target	F%	Out	Target	F%	Out	Target	F%	Out	
Coliform bacteria (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	1	7	<i>C. freundii</i> [<i>A. hydrophila</i>]	4	0	<i>E. coli</i> <i>C. sakazakii</i>	4	7	
Susp. thermo-tolerant colif. bact. (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	—	—	[<i>C. freundii</i>]	—	—	<i>E. coli</i> [<i>C. sakazakii</i>]	—	—	
<i>E. coli</i> (MF)	<i>E. coli</i> [<i>K. pneumoniae</i>]	10	0	—	1	—	{ <i>E. coli</i> }	21	0	
Coliform bacteria (rapid method)	<i>E. coli</i> <i>K. pneumoniae</i>	0	2	<i>C. freundii</i>	2	2	<i>E. coli</i> <i>C. sakazakii</i> <i>S. marcescens</i>	0	2	
<i>E. coli</i> (rapid meth.)	<i>E. coli</i>	0	2	—	2	—	<i>E. coli</i>	0	4	
Presumptive <i>C. perfringens</i> (MF)	—	2	—	<i>C. perfringens</i>	0	0	—	2	—	
<i>Clostridium perfringens</i> (MF)	—	0	—	<i>C. perfringens</i>	0	0	—	0	—	
Moulds (MF)	<i>Ph. malorum</i>	5	0	—	0	—	—	7	—	
Yeasts (MF)	<i>K. marxianus</i>	0	5	—	2	—	—	19	—	
Culturable micro-organisms (total count), 3 days	22 °C	<i>S. xylosus</i> (<i>E. coli</i>) (<i>K. pneumoniae</i>)	0	5	<i>C. freundii</i> <i>A. hydrophila</i>	1	2	<i>S. cohnii</i> (<i>E. coli</i>) (<i>C. sakazakii</i>) (<i>S. marcescens</i>)	0	0

* In total 44 out of 95 laboratories (46%) reported at least one deviating result

— Organism or numerical result is missing

() The organism contributes with only very few colonies

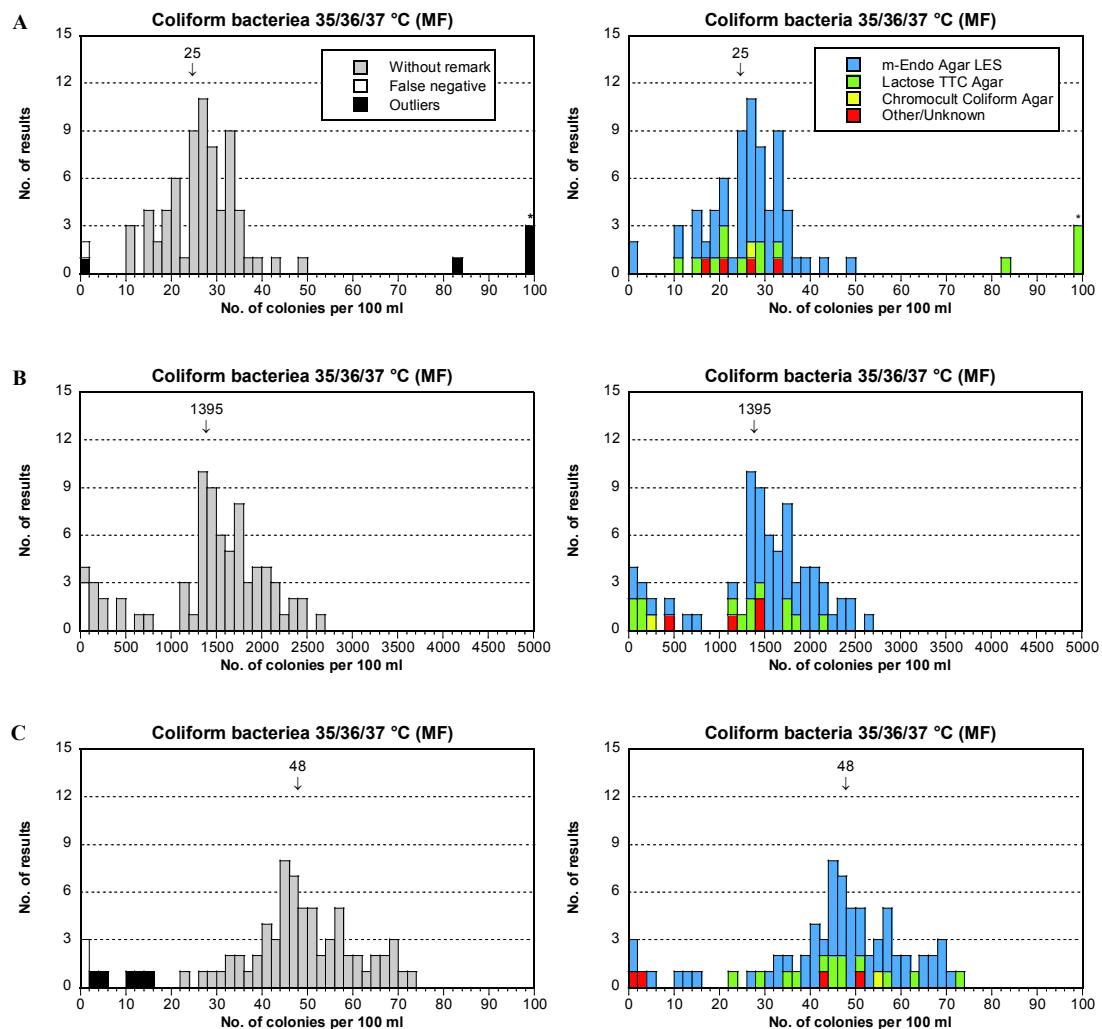
[] The organism is false positive on the primary growth medium

{ } The organism may give different results depending on the methods or definitions used

Coliform bacteria (MF)

Even though the reporting of method data happened not to be mandatory for the analysis of coliform bacteria, most of the laboratories did report it. In those cases where method data was missing, we have used the data valid for the laboratory during their last participation to a PT round – after giving the laboratories possibility to change it.

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	n	Mv	CV	F	<	n	Mv	CV	F	<	>		
Total	75	69	25	15	1	1	4	72	1395	25	3	0	0	67	48	12	3	5	0
m-Endo Agar LES	57	55	26	15	1	1	0	56	1500	22	1	0	0	51	49	11	2	4	0
Lactose TTC Agar	13	9	21	17	0	0	4	11	1171	33	2	0	0	13	45	15	0	0	0
Chromocult C Agar	1	1	27	–	0	0	0	1	200	–	0	0	0	1	54	–	0	0	0
Other/Unknown	4	4	23	–	0	0	0	4	1050	–	0	0	0	2	46	–	1	1	0



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

From the table it is clear that more than 4 times as many laboratories are using LES compared to LTTC. The results indicate somewhat lower results with LTTC than with LES, for all mixtures. The dispersion, and thus the uncertainty, is simultaneously somewhat higher for LTTC. It is difficult to find clear differences, although there are tails with low results in both mixture B and C.

Mixture A

- *E. coli* and *K. pneumoniae* form colonies in the analysis of coliform bacteria. The colonies are typical with a metallic sheen on LES. The reading of colonies is a bit more difficult on LTTC at 37 °C because *S. xylosus* grows as background. At least it was possible to count the target colonies from the volume 10 ml. As often, it was difficult to distinguish the yellow colour in the medium for individual colonies, as the whole medium turns yellow due to a lot of target colonies present.
- All four high outliers were obtained with LTTC and might be due to the growth of *S. xylosus*, although it forms atypical colonies. No particular problem based on method seems otherwise to occur.

Mixture B

- The colonies of both *C. freundii* and *A. hydrophila* are typical for suspected coliform bacteria on both LES and LTTC, but with somewhat different appearance. The yellow colour beneath the colonies in LTTC is, as usual, difficult to distinguish.
- *A. hydrophila* is oxidase positive. The colonies can thus be excluded from coliform bacteria after confirmation by the oxidase test.
- A relatively large number of low results was obtained that could not be identified as outliers, with the exception of 3 false negative results. These low results cause a lower total mean value than when the rapid method Colilert Quanti-Tray® was used. The low values might be caused by counting a smaller volume but forgetting to calculate the result for the volume 100 ml.

Mixture C

- The strains of *E. coli*, *C. sakazakii* and *S. marcescens* grow all on the media for coliform bacteria. *S. marcescens* ferment lactose badly and give rise to atypical colonies, i.e. green on LTTC and red on LES. That strain will most certainly not be included as suspected coliform bacterium on media based on lactose fermentation, and will not be selected for confirmation. The colonies of the other two strains are, however, typical for the various media. Negative oxidase tests will show that they are coliform bacteria.
- An unusually large number of deviating results were obtained without a clear cause.

Suspected thermo-tolerant coliform bacteria (MF)

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

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

Standard, Method	Tot n	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
Total	42	42	24	—	—	—	42	0	—	—	—	42	36	—	—	—
EN ISO 9308-1	6	6	20	—	—	—	6	0	—	—	—	6	37	—	—	—
SS 028167	13	13	27	—	—	—	13	0	—	—	—	13	33	—	—	—
SFS 4088	16	16	26	—	—	—	16	0	—	—	—	16	37	—	—	—
NS 4792	7	7	20	—	—	—	7	0	—	—	—	7	31	—	—	—
Other/Unknown	0	-	-	—	—	—	-	-	—	—	—	-	-	—	—	—

The Swedish standard states incubation at 44 °C but one laboratory used 44.5 °C. The temperature 44 °C is also stated in EN ISO 9308-1. One laboratory using Norwegian standard has incubated at 44 °C while all the others have used 44.5 °C. All laboratories using Finnish standard reported 44 °C.

In both mixture A and C, a lower average was obtained by the 7 laboratories using Norwegian standard, NS 4792, compared to most other groups. Probably, it is caused by the fact that 6 of the laboratories incubated at 44.5 °C, since the average for the 7 laboratories incubating at 44.5 °C was 18 cfu/100 ml in mixture A and 27 cfu/100 ml in mixture C. For unknown reason, also laboratories using LTTC at 44 °C in mixture A obtained lower results than laboratories using m-FC with Swedish or Finnish standard.

Mixture A

- *K. pneumoniae* grows together with *E. coli* on m-FC at 44 °C but appears with more light blue colonies.
- Probably, the growth of *K. pneumoniae* is the most hampered at 44.5°C.

Mixture B

- No suspected thermo-tolerant coliform bacteria were present in the mixture.

Mixture C

- Beside the strain of *E. coli*, *C. sakazakii* grow with greyish colonies at 44 °C. It is not clear which strain is the most hampered at 44.5 °C.

Escherichia coli (MF)

E. coli is quantified after confirmation of colonies that have grown either at 36±2 °C or at 44/44.5 °C. Different primary growth media are used at the two temperatures, LTTC or LES at the low temperature and LTTC or m-FC at the high temperature. The results from the two temperatures are here compiled in separate tables. In some cases, the incubation temperature stated for the primary growth medium was ambiguous. These results, as well as the deviating results for this group, are not specifically given but are only included in the table "All results".

E. coli was present in mixture A and C. The average of accepted results is lower for LTTC than for LES in both these mixtures at 36±2 °C. Besides, there are 4 false negative results for LTTC in mixture A without any obvious reason. In mixture C there are more false negative results for LES than for LTTC, but here is a plausible explanation (see below under mixture A).

No method differences could be seen for any mixture at 44/44.5 °C. The numbers of results are too few. However, some false negative results were obtained.

All results

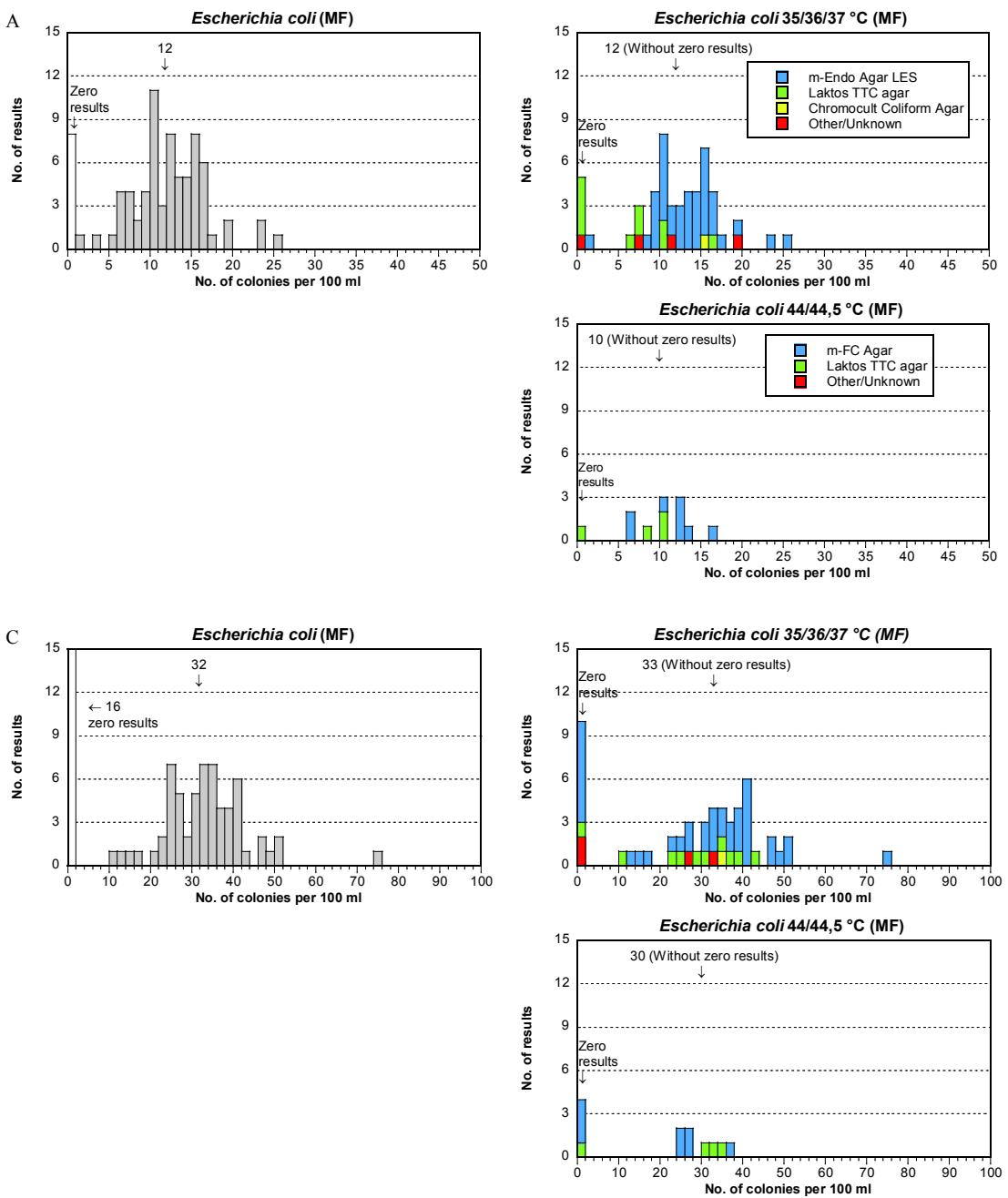
Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	77	69	12	20	8	0	0	76	0	-	1	-	-	61	32	16	16	0	0

From 36±2 °C

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	53	48	12	19	5	0	0	52	0	-	1	-	-	43	33	17	10	0	0
m-Endo Agar LES	38	38	13	18	0	0	0	37	0	-	1	-	-	31	35	17	7	0	0
Lactose TTC Agar	10	10	9	19	4	0	0	10	0	-	0	-	-	9	29	18	1	0	0
Chromocult C Agar	1	2	0	-	0	0	0	1	0	-	0	-	-	1	35	-	0	0	0
Other/Unknown	4	1	0	-	1	0	0	4	0	-	0	-	-	2	29	-	2	0	0

From 44/44.5 °C

Medium/Standard	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	12	11	10	15	1	0	0	12	0	-	0	-	-	8	30	7	4	0	0
<i>Medium</i>																			
m-FC Agar	8	8	11	17	0	0	0	8	0	-	0	-	-	5	28	8	3	0	0
Lactose TTC Agar	4	3	9	6	1	0	0	4	0	-	0	-	-	3	33	4	1	0	0
Other/Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<i>Standard</i>																			
EN ISO 9308-1	4	3	9	-	1	0	0	4	0	-	0	-	-	3	33	-	1	0	0
SS 028167	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
SFS 4088	2	2	11	-	0	0	0	2	0	-	0	-	-	2	26	-	0	0	0
NS 4792	4	4	9	-	0	0	0	4	0	-	0	-	-	2	31	-	2	0	0
Other/Unknown	2	2	14	-	0	0	0	2	0	-	0	-	-	1	25	-	1	0	0



Mixture A

- A confirmation step was needed irrespectively if *E. coli* was quantified from the primarily analysis at 36 ± 2 or $44/44.5$ °C. The strains of *E. coli* and *K. pneumoniae* grow at both temperatures. *K. pneumoniae* is excluded as presumptive *E. coli* due to its lack of indol production and β -glucuronidase activity.
- There is no clear reason to the 8 false negative results. Six of them are from LTTC.

Mixture B

- No *E. coli* was included in the mixture.

Mixture C

- Confirmation is needed when *E. coli* is quantified from the primary analysis at 36 ± 2 °C. Both the strain of *E. coli* and *C. sakazakii* grow as typical coliform bacteria. *C. sakazakii* will be excluded as presumptive *E. coli* due to its lack of indol production and β -glucuronidase activity.
- Both strains will also grow at 44/44.5 °C but the colonies of *C. sakazakii* are atypical, greyish on m-FC. Colonies of *C. sakazakii* suspected as presumptive *E. coli*, e.g. on LTTC, will be excluded after confirmation, as described above.
- The strain of *E. coli* present does not produce gas while fermenting lactose. 31 laboratories reported that they have used gas production test in the confirmation step. 13 of these have reported a zero result together with three laboratories not stating the use of gas production test. This is the explanation to the majority of the 16 zero results.
- If gas production is a criterion for *E. coli*, the zero results based on the lack of gas production should not be reckoned as false negative ones.
- It seems that 18 of the 31 laboratories reporting the use of gas production did not use the test result as a confirmation criterion for *E. coli*.

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

The rapid method used for both these parameters was almost exclusively Colilert® Quanti-Tray® from the manufacturer IDEXX Inc. Two laboratories have not used a rapid method but the classical multiple tube method with MPN quantification (Standard Methods 9221B; 5). Of the 53 laboratories that reported Colilert some used the trays with 51 wells, while others used trays with 97 wells (a few of which, probably incorrectly, have reported 96 wells). Only one laboratory did not indicate which kind of trays they used. The laboratories have often analysed both diluted and undiluted samples.

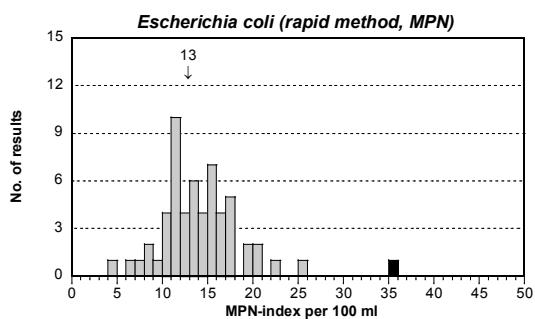
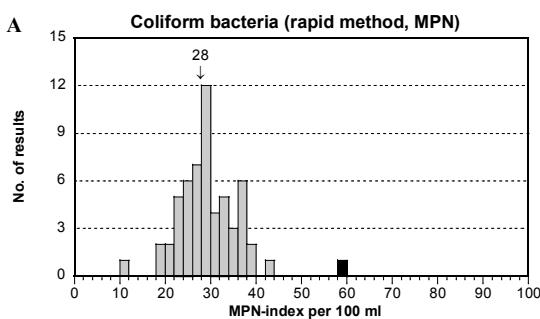
Neither for coliform bacteria nor for *E. coli* were differences obvious in any of the mixtures based on the trays used. Only 1–2 deviating result were reported per mixture.

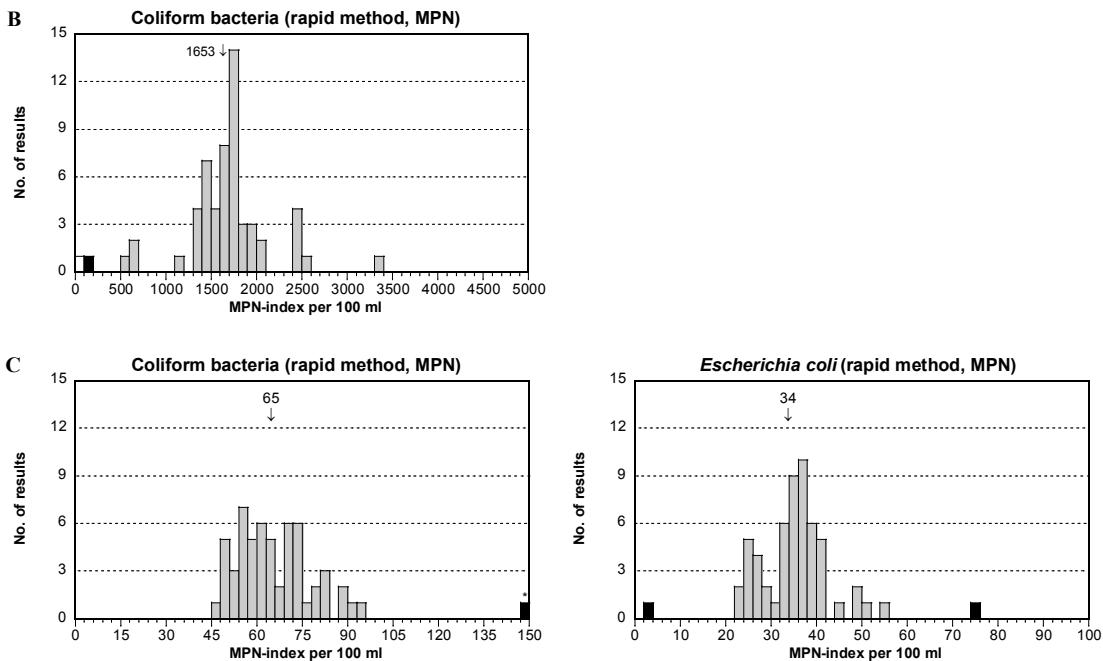
Coliform bacteria, Rapid method with MPN

Medium	Tot n	A					B					C							
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >			
Total, Rapid meth.	55	54	28	11	0	0	1	53	1684	13	1	1	0	54	65	9	0	0	1
Colilert Quanti-51	13	13	31	11	0	0	0	13	1539	12	0	0	0	13	64	11	0	0	0
Colilert Quanti-97	39	38	27	10	0	0	1	38	1742	13	1	0	0	38	66	9	0	0	1
Colilert Quanti-?	1	1	35	-	0	0	0	1	1730	-	0	0	0	1	56	-	0	0	0
Other/Unknown	2	2	34	-	0	0	0	1	1400	-	0	1	0	2	57	-	0	0	0
Not rapid method	2	2	28	-	0	0	0	2	920	-	0	0	0	2	55	-	0	0	0

E. coli, Rapid method with MPN

Medium	Tot n	A					B					C						
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >		
Total, Rapid meth.	56	55	13	15	0	0	1	55	0	-	1	-	54	35	10	0	1	1
Colilert Quanti-51	14	14	15	18	0	0	0	14	0	-	0	-	14	33	9	0	0	0
Colilert Quanti-97	39	38	13	14	0	0	1	38	0	-	1	-	37	35	10	0	1	1
Colilert Quanti-?	1	1	12	-	0	0	0	1	0	-	0	-	1	37	-	0	0	0
Other/Unknown	2	2	14	-	0	0	0	2	0	-	0	-	2	35	-	0	0	0
Not rapid method	2	1	17	-	0	0	0	2	0	-	0	-	1	22	-	0	0	0





Mixture A

- Both *E. coli* and *K. pneumoniae* are typical coliform bacteria, producing β -galactosidase and detected by methods based on the activity of this enzyme (ONPG positive), e.g. Colilert®-18/24 Quanti-Tray® where ONPG is a substrate.
- Only the *E. coli* strain produces β -glucuronidase and is detected as *E. coli* by methods based on the activity of this enzyme.
- The average results for both coliform bacteria and *E. coli* were slightly higher with Colilert®-18/24 Quanti-Tray® compared to with the MF-method and had fewer deviating results, as is often the case.

Mixture B

- *C. freundii* was detected as a coliform bacterium based on the activity of the enzyme β -galactosidase. The growth of *A. hydrophila* is inhibited by the antibiotics in the medium. The average result was somewhat higher with Colilert compared to the MF-method.
- No *E. coli* was present in the mixture. One false positive result was reported.

Mixture C

- All the strains *E. coli*, *C. sakazakii* and *S. marcescens* produce the enzyme β -galactosidase and contribute to the result of coliform bacteria obtained with Colilert®-18/24 Quanti-Tray®. The average for coliform bacteria was therefore clearly higher with this method (65 cfu/100 ml) than with the MF-method (48 cfu/100 ml), where *S. marcescens* is not reckoned as a coliform bacterium.
- Only the *E. coli* strain in the mixture is β -glucuronidase positive, and is detected as *E. coli* with Colilert®-18/24 Quanti-Tray®. The average for accepted values is similar to corresponding average for the MF-method.

Clostridium perfringens (MF)

The analysis of *Clostridium perfringens* is performed differently in different countries and laboratories, because no international standard is stated as reference method in the European Drinking Water Directive (4). The parameter to be analysed according to the directive is spores and vegetative cells of *C. perfringens*. In Sweden, the result of **presumptive *C. perfringens*** is also often accepted and therefore accounted for separately.

There is one method explicitly described in the Drinking water directive (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) was accepted by the responsible group under the EU Commission. Adjustments in the draft approved in ISO meetings have been pointed out in the instructions for the proficiency testing round. The standard has now the number ISO/DIS 14189 (DIS = Draft International Standard) and should be finally established in 2013 with the number ISO 14189. It is basically equivalent to the CD version from 2002 after adjustments but has now a much more simplified confirmation step. In the new version, isolated colonies are only tested for activity of the enzyme acid phosphatase.

Some other methods/media were used by individual participants, such as the Danish standard DS 2256 with Iron Sulfite agar and EN ISO 26461-2:1993 for analysis of spores only (heating of the sample). The last method has the same base medium (PAB/TSC) as in ISO/CD 6461-2 and ISO/DIS 14189, but without any confirmation.

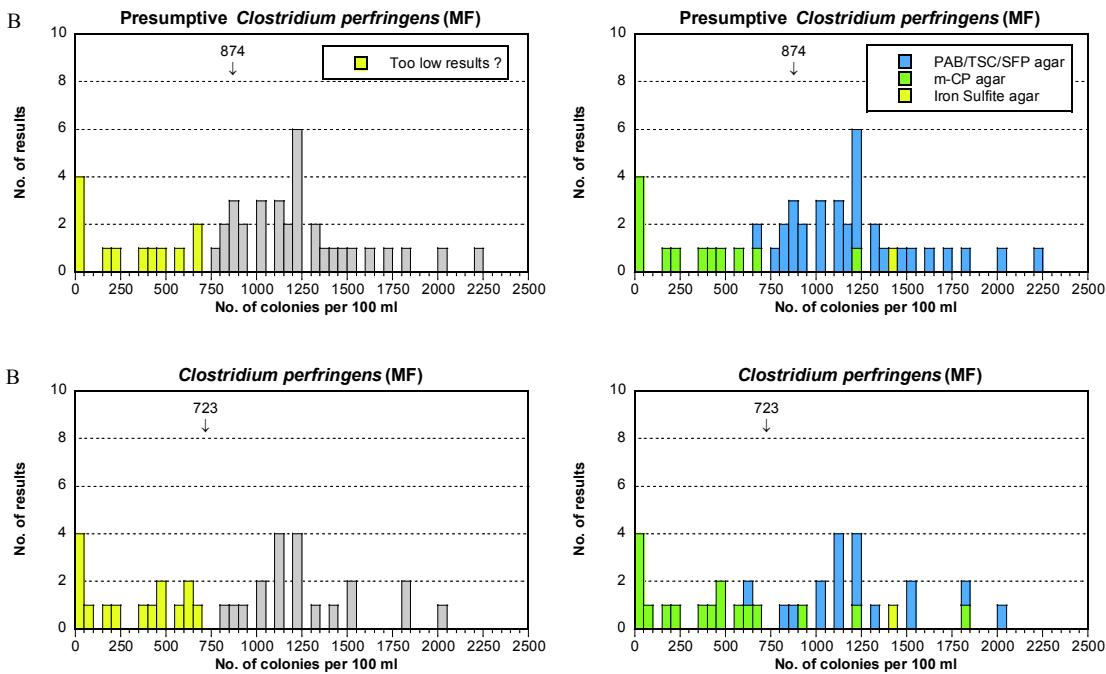
Both presumptive *C. perfringens* and *C. perfringens* analyses show that the yield with m-CP Agar is much lower with the strain present in the mixture compared with other media. The histograms show that the low results are almost exclusively obtained with m-CP Agar. Very few results were present for m-CP Agar with concentration as for PAB/TSC/SFP.

Presumptive Clostridium perfringens MF

Medium	Tot n	A					B					C					
		n	Mv	CV	F	<	n	Mv	CV	F	<	n	Mv	CV	F	<	>
Total	45	44	0	-	1	-	45	874	36	0	0	0	44	0	-	1	-
PAB/TSC/SFP agar	32	31	0	-	1	-	32	1185	14	0	0	0	31	0	-	1	-
m-CP agar	12	12	0	-	0	-	12	254	65	0	0	0	12	0	-	0	-
Iron Sulfite agar	1	1	0	-	0	-	1	1400	-	0	0	0	1	0	-	0	-

Clostridium perfringens MF

Medium	Tot n	A					B					C					
		n	Mv	CV	F	<	n	Mv	CV	F	<	n	Mv	CV	F	<	>
Total	35	35	0	-	0	-	35	723	44	0	0	0	35	0	-	0	-
PAB/TSC/SFP agar	17	17	0	-	0	-	17	1183	14	0	0	0	17	0	-	0	-
m-CP agar	17	17	0	-	0	-	17	352	61	0	0	0	17	0	-	0	-
Iron Sulfite agar	1	1	0	-	0	-	1	1400	-	0	0	0	1	0	-	0	-



Mixture A and C

- No *C. perfringens* or other presumptive *C. perfringens* was present in the mixture.

Mixture B

- A strain of *C. perfringens* was present. Different results were obtained depending on the method used (see tables and figures).
- The colour of the colonies with PAB/TSC/SFP may vary from light grey or yellowish-brown to completely black, at least partly depending on the condition of the medium and its reduction potential.
- The distribution of the results is very wide and atypical compared to other parameters. Because of this it is almost impossible to identify outliers. However, the results obtained with PAB/TSC/SFP only are better distributed.
- In comparison with PAB/TSC/SFP, most results obtained with m-CP Agar and the *C. perfringens* strain present can be considered as abnormally low.

Moulds and yeasts (MF)

Out of the 43 laboratories that analysed moulds and yeasts, 35 reported the use of the Swedish standard SS 028192. Besides Sweden it is used in Denmark but also in Finland and Norway under their own national designations SFS 5507 and NS 4716, respectively. Eight laboratories used other methods, such as a food method from NMKL and a method in Standard Methods of Water and Wastewater (5).

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, "Rose Bengal Chloramphenicol" (RBC) agar and "Dichloran Rose Bengal Chloramphenicol" (DRBC) agar. 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. Sometimes only one of them are reported as used, which then is in accordance with what is recommended from manufacturers of the respective medium. Many laboratories using a commercial medium in which Rose Bengal is included have not reported the use of Rose Bengal. What are base medium and supplements compared to finished medium? The information given for the media and inhibitors used are in many cases not clear. The Swedish laboratories are usually using chlortetracycline and chloramphenicol together in the Rose Bengal agar, as is stated in the standard SS 028192. This medium is here designated RBCC agar from the English spelling of the characteristic ingredients.

Some unusual media such as Dichloran Glycerol 18% (DG 18) and "Malt Extract Agar" (MEA) were used together with some odd methods. The standard ISO 7954 (1987) use the medium Glucose Yeast Extract agar with Oxytetracycline as inhibiting substance (OGYE).

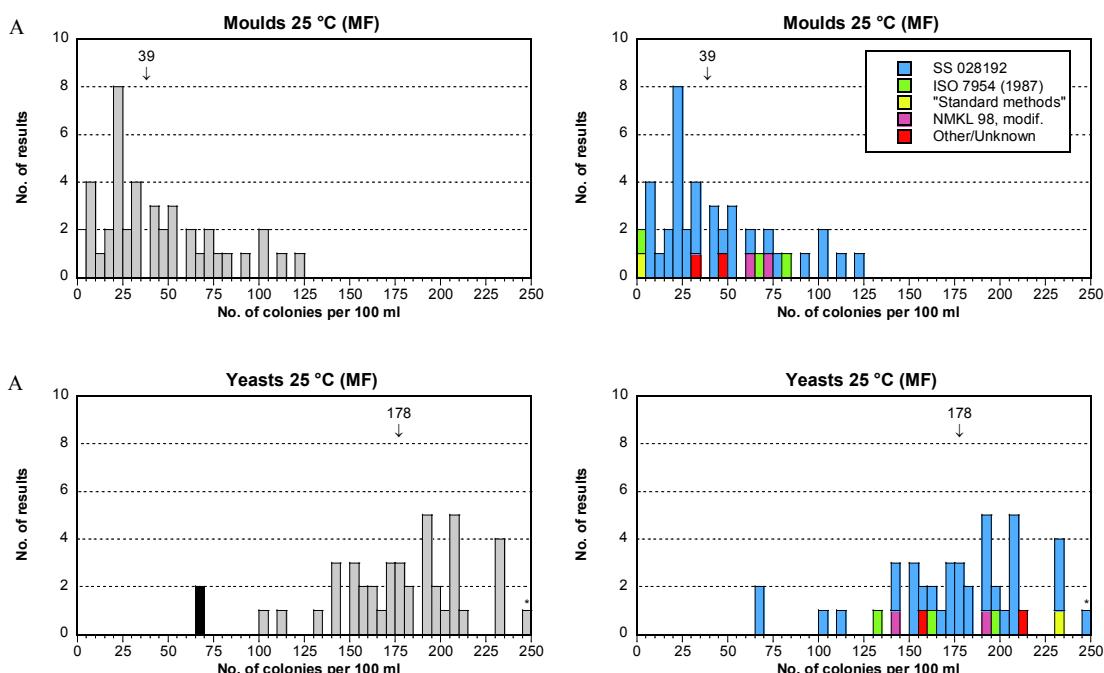
Moulds MF

Standard, Method	Tot n	A					B					C					
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >	
Total	43	41	39	37	2	0	0	42	0	-	0	-	39	0	-	3	-
SS 028192	35	35	36	39	0	0	0	34	0	-	0	-	31	0	-	3	-
ISO 7954 (1987)	3	2	72	7	1	0	0	3	0	-	0	-	3	0	-	0	-
Standard methods	1	0	-	-	1	-	-	1	0	-	0	-	1	0	-	0	-
NMKL 98, Modif.	2	2	65	-	0	0	0	2	0	-	0	-	2	0	-	0	-
Other/Unknown	2	2	38	-	0	0	0	2	0	-	0	-	2	0	-	0	-

Yeasts MF

Standard, Method	Tot n	A					B					C					
		n	Mv	CV	F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	< >	
Total	43	41	178	10	0	2	0	41	0	-	1	-	34	0	-	8	-
SS 028192	35	33	179	10	0	2	0	33	0	-	1	-	27	0	-	7	-
ISO 7954 (1987)	3	3	161	10	0	0	0	3	0	-	0	-	2	0	-	1	-
Standard methods	1	2	230	-	0	0	0	1	0	-	0	-	1	0	-	0	-
NMKL 98, Modif.	2	2	164	-	0	0	0	2	0	-	0	-	2	0	-	0	-
Other/Unknown	2	2	181	-	0	0	0	2	0	-	0	-	2	0	-	0	-

The standard SS 028192 is the only one used by more than 3 laboratories and a further separation into groups within that standard is very uncertain. For example, no evident differences can be seen depending on the different incubation conditions. The temperature is in almost all cases 25 °C and the time 7 days as specified in SS 028192. Therefore, it is not meaningful to discuss differences among results from the tables, neither for yeasts nor for moulds. The histograms show generally that the results from the odd methods are scattered among those obtained by use of SS 028192. However, the 2 false negative mould results came from these odd methods.



Mixture A

- The mould *Phialophora malorum* and the yeast *Kluyveromyces marxianus* were present in the mixture. Apart from a few individual deviating results, the analysis did not cause any problem. Although the results were scattered, the distributions could be considered as quite good for fungal analyses.

Mixture B and C

- No moulds and yeasts were present in the mixtures. Mixture B did not cause any problem, with some exceptions. On the other hand, three false positive results for moulds and 8 for yeasts were reported for mixture C. The yeast results can be explained by the fact that the bacterium *S. marcescens* can form reddish colonies on the fungal media, which can be misjudged as yeast colonies without microscopically observation. However, there is no certain explanation for the reported mould colonies. Individual mould colonies may appear due to deposition of spores from the laboratory air during the analysis. Such a result may not necessarily be interpreted as false positive for the sample needing follow-up actions, even though the result is false positive in itself.

Culturable microorganisms 22 °C, 3 days

Out of more than 90 laboratories only 3 reported another method than XX-EN ISO 6222:1999. These 3 laboratories did not report any outlier.

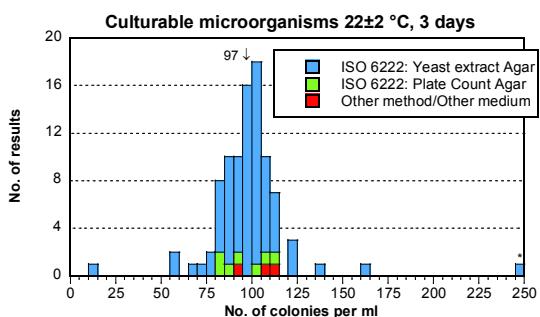
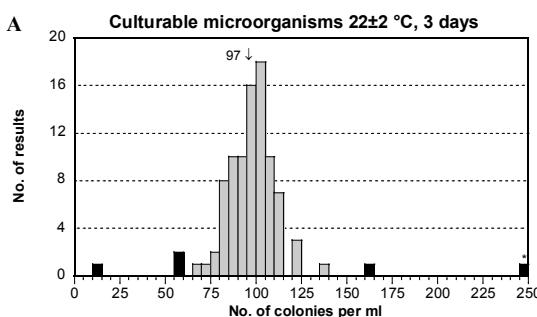
Only comparisons of method variants of the XX-EN ISO 6222:1999 are therefore relevant to discuss. Here, results are presented according to culture media and magnification for reading.

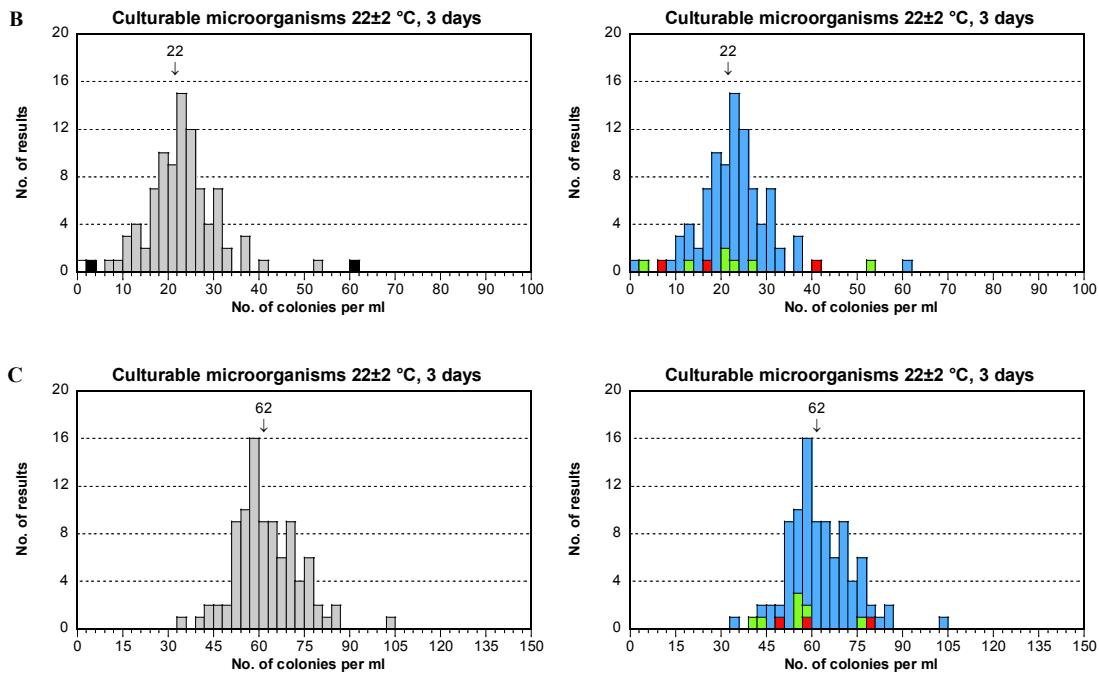
No general pattern can be discerned in terms of medium. For mixture C there is a trend of slightly lower results with Plate Count Agar (PCA) compared to Yeast extract Agar (YEA). The CV of the results is largest with PCA in mixture B.

Deviating results are mainly obtained while using low magnification.

22±2 °C, 3 days

Group of results	Tot n	A					B					C							
		n	Mv	CV	F	<	n	Mv	CV	F	<	n	Mv	CV	F	<	>		
Total, all results	92	87	97	6	0	3	2	89	22	17	1	1	92	62	9	0	0	0	
EN ISO 6222	89	84	97	6	0	3	2	86	22	16	1	1	89	62	9	0	0	0	
<u>Medium</u>																			
Yeast extract Agar	82	77	97	6	0	3	2	80	22	15	1	0	1	82	62	8	0	0	0
Plate Count Agar	7	7	95	6	0	0	0	6	24	26	0	1	0	7	54	11	0	0	0
Other/Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<u>Magnification</u>																			
None	28	26	97	7	0	1	1	25	20	17	1	1	1	28	60	8	0	0	0
1,1–4,9×	28	26	98	5	0	2	0	28	23	18	0	0	0	28	65	9	0	0	0
5–11,9×	32	31	96	6	0	0	1	32	23	11	0	0	0	32	60	8	0	0	0
> 12×	1	1	82	-	0	0	0	1	21	-	0	0	0	1	55	-	0	0	0
Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Other method	3	3	103	-	0	0	0	3	18	-	0	0	0	3	61	-	0	0	0





Mixture A

- The three bacterial strains formed colonies in proportion linked to their concentration in the mixture. *S. xylosus* was the most abundant.
- Except for 5 outliers the analysis did not cause any problem. The distribution of the results was good.

Mixture B

- Only *C. freundii* and *A. hydrophila* formed colonies.
- The distribution was good with the exception of the 3 deviating results.

Mixture C

- All strains present in the mixture grow as culturable microorganisms at 22 °C. Colonies of the strain of *S. cohnii* were most abundant.
- No deviating results were identified. The distribution of the results was good.

Outcome of the results and laboratory assessment

General information about reported results

All laboratories' reported results are compiled in **annex A**. 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 a particular laboratory to summarize its performance. These values are highlighted with bold text and colour background in annex A. The limit values for lowest and highest accepted results are given in the summarizing lines at the end of annex A, together with the measurement uncertainty of the mean.

When it is evident that a laboratory has mixed up results it is mentioned in the text. If whole samples seem to have been mixed up, the respective sample numbers are hatched in annex A. No mixing up seem to have occurred this time, neither of individual results nor of the whole samples. In some cases, however, it can be suspected that the laboratory has forgotten to calculate the results for the volume asked for, i.e. 100 ml for all analyses except for culturable microorganisms where 1 ml is appropriate.

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 annex A.

Assessment of the performance

The laboratories are not grouped or ranked in relation to their performances. The assessment is basically a clear description of the number of false results and outliers.

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

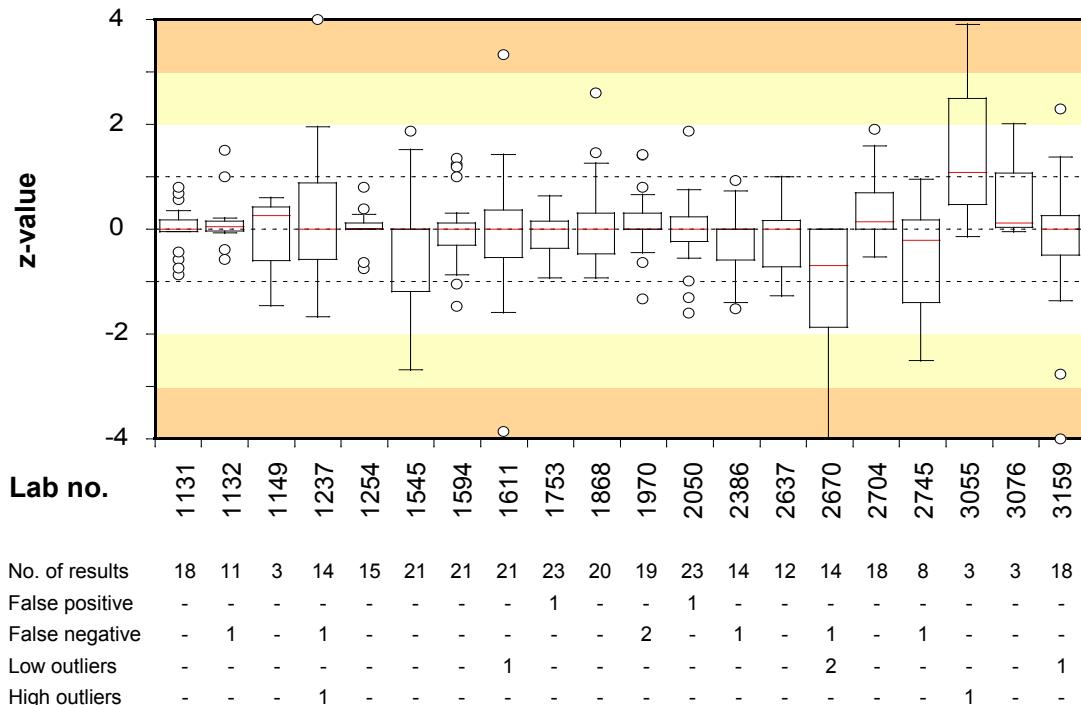
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are shown in **annex B** but are not evaluated more elaborately. They are given explicitly to facilitate the follow-up process for the laboratories using z-scores.

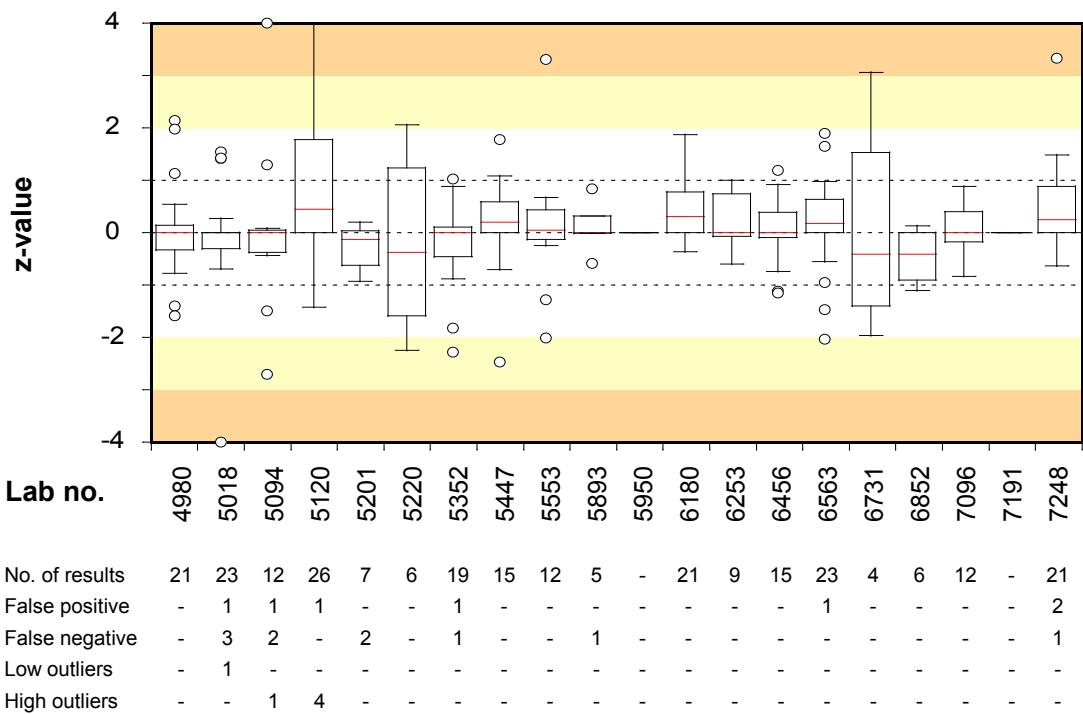
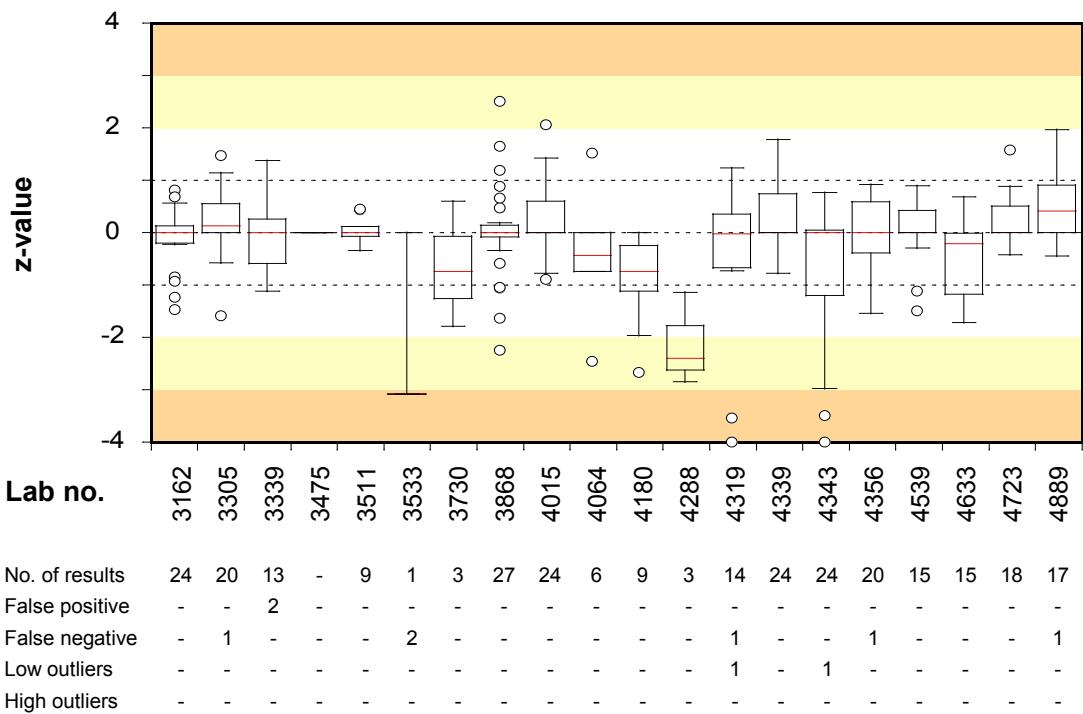
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

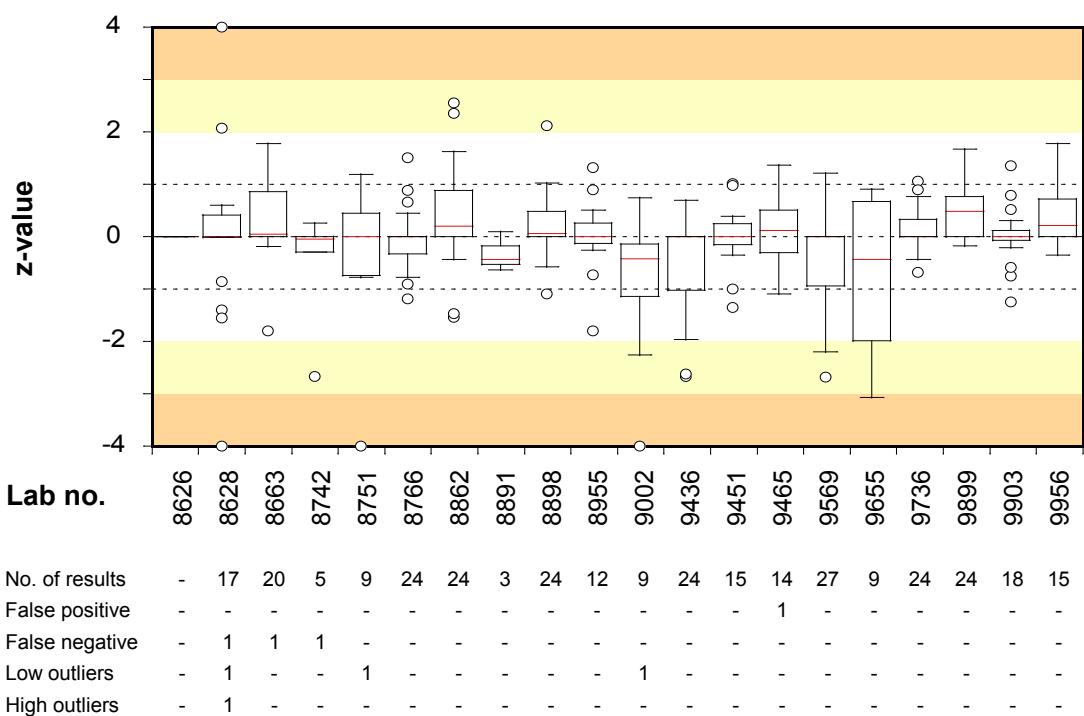
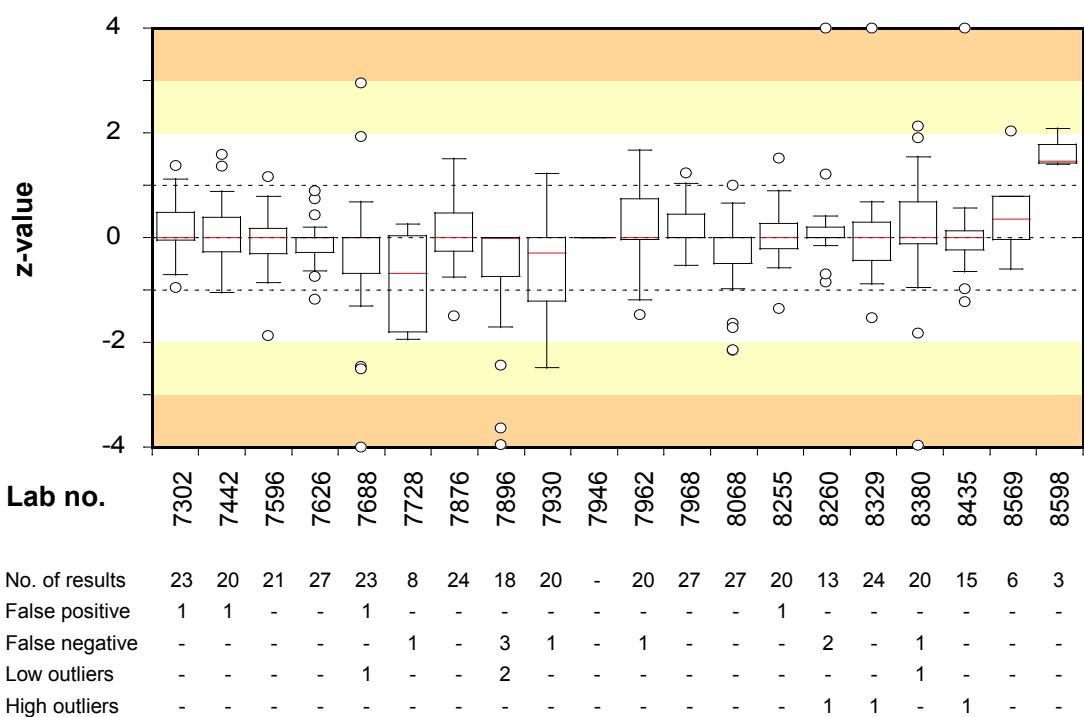
Box plots and numbers of deviating results for each participating laboratory

- Z-scores are calculated from the formula $z = (x - mv) / s$
- Z-scores $> +4$ and < -4 have been set to $+4$ and -4 , respectively.
- False results do not generate z-scores and are not included in 'No. of results'. False positive results cannot be illustrated in the box plots.
- The outliers are included in the plots after recalculation to standardised values with the same s values as the rest of the results.
- The numbers of false positives and false negatives are given in the table under the plots together with the numbers of outliers.
- The horizontal red line in each box indicates the median for the laboratory.
- The box includes 25% of the results above and below the median. The lines protruding from the box and/or the circles embrace the remaining 50% of the results, false results excluded.
- A circle is shown when a result is highly deviating* from the rest.
- The background is divided into coloured fields in order to simplify localization of the laboratory results.

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







Test material, quality controls and processing of data

Test material and its content

The test material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd (2). This round comprised three test items with different microorganism mixtures. Each laboratory received one vial of each mixture. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition and concentrations of each mixture is listed in **Table 2**. The participating laboratories were assigned to perform the analyses according to the methods routinely used on drinking water samples.

Table 2 Microorganisms present in the mixtures

Mixture ¹	Microorganisms	Strain no.	cfu/100 ml ²
A	<i>Escherichia coli</i>	SLV-165	13
	<i>Klebsiella pneumoniae</i>	SLV-537	17
	<i>Kluyveromyces marxianus</i>	SLV-439	148
	<i>Phialophora malorum</i>	SLV-545	52
	<i>Staphylococcus xylosus</i>	SLV-283	100*
B	<i>Citrobacter freundii</i>	SLV-424	1800
	<i>Aeromonas hydrophila</i>	SLV-081	1500
	<i>Clostridium perfringens</i>	SLV-442	730
C	<i>Escherichia coli</i>	SLV-532	38
	<i>Cronobacter sakazakii</i>	SLV-419	10
	<i>Serratia marcescens</i>	SLV-040	22
	<i>Staphylococcus cohnii</i>	SLV-462	64*

1 The links between the mixtures and the randomised sample numbers are shown in annex A

2 cfu = colony forming units; * indicates cfu per ml; at the times given in note 1 of table 3

Quality control of the mixtures

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 in 11 to 13 vials of each mixture and the biggest differences between vials were 3, 3 and 4 mg for mixture A, B and C, respectively. The highest accepted volume variation is 15 mg (3%). **Table 3** presents the results from the organizer in the form of concentration means (cfu) and coefficients of variation (CV) from duplicate analyses of 10 vials from each mixture. The results relate to the unit by volume at which the colonies were in fact counted. According to the criteria for the CVs used they were acceptable for the mixtures to be considered homogenous. The highest accepted CV normally is 25%. For very low colony counts a higher CV is accepted.

Table 3 Contents (cfu) and measures of homogeneity (CV; coefficient of variation in per cent) in relevant sample volumes for the various parameters in the mixtures¹

Analysis parameter	Mixture					
	Method standard for analysis		A		B	
	cfu	CV	cfu	CV	cfu	CV
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	30	5	18	10 ^b	48	5
Suspected thermo-tolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	28	8	—	—	44	5 ^c
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	13	7	—	—	38	6
Presumptive <i>Clostridium perfringens</i> (MF) <i>TSC Agar according to ISO/CD 6461-2:2002</i>	—	—	73	6 ^a	—	—
Moulds (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	5	20 ^a	—	—	—	—
Yeasts (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	15	8 ^a	—	—	—	—
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	100	5	39	4	65	3

1 n=10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, 7, 8 and 9 weeks ahead of the testing round start for the mixtures A, B and C, respectively

a Result for 10 ml

b Result for 1 ml

c Including both *E. coli* and *C. sakazakii*

— No target organism

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 which give better normal distributions and therefore decrease the significance of the “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 used as risk to incorrectly assess a result as being an outlier. Although the method is objective, it 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 as a false negative result. In special situations, e.g. when many zero results are reported and in some borderline cases, a few subjective

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

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

The calculation of uncertainty of measurement of the assigned value is described in the scheme protocol (1). The assigned value for an analysis is calculated from the square root transformed results and is the square root of "Mean" in Annex A, and there denoted as mv . Hence, also the measurement uncertainty will be expressed in the square root form. 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 multiplication by 100.

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

Processing of methods data

The test material is primarily adapted to the EN ISO methods for analyses of drinking water referred to in the European Drinking water directive (4). Alternative methods acknowledged within EU may normally also be used without any problem, as is also the case with many other methods.

To be able to describe the results as fair as possible the results are grouped based on used method variant. The requirements to group results are found in the standard EN ISO/IEC 17043:2010, for which the proficiency testing schemes of the National Food Agency is accredited. This is the reason why it is mandatory for the participants to give information about the methods used in the analyses for which they send in results and that will be evaluated for performance. The method information can be reported after logging in on our website www.slv.se/absint.

References

1. Anonymous 2012. Scheme protocol, Microbiology, Drinking water & Food. The National Food Agency, Sweden.
2. Peterz, M., Steneryd, A.-C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. J. Appl. Bacteriol. 74:143-148.
3. Kelly, K. 1990. Outlier detection in collaborative studies. J. Assoc. Off. Chem. 73:58-64.
4. Anonymous 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal of the European Communities. 5.12.98, L 330/32-54 (*national translations available*).
5. Standard Methods for the Examination of Water and Wastewater,
<http://www.standardmethods.org/>

Annex A Results of the participants. Susp. = suspected on membrane filter before confirmation. Results given as <1, <2, <10 and <100 are treated as zero. The fields with other results given as < 'value' and results given as > 'value' are yellow, and those results are not included in calculations or evaluations, 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	1 2 3	30	1920	48	30	1410	48	-	-	-	12	0	24	28	1733	73	11	0	37
1132	1 3 2	-	-	-	-	-	-	28	0	38	12	0	0	26	2420	64	11	0	36
1149	2 3 1	21	1330	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	3 2 1	-	-	-	10500	1700	45	-	-	-	0	0	30	19	1300	65	11	0	41
1254	2 1 3	32	2000	50	32	1600	50	20	0	36	12	0	25	-	-	-	-	-	-
1545	3 2 1	18	185	27	18	147	27	18	0	27	6	0	21	-	-	-	-	-	-
1594	1 3 2	18	2400	47	18	2400	47	25	1000	36	12	0	47	20	1800	55	12	0	34
1611	2 3 1	31	2100	44	31	1500	44	21	0	28	15	0	40	20	1414	62	11	0	26
1753	1 3 2	29	1714	39	29	1714	39	-	-	-	11	0	27	27	1961	59	11	0	36
1868	3 2 1	26	1300	55	26	1300	55	-	-	-	9	0	46	26	1658	83	10	0	55
1970	1 2 3	32	2200	53	32	1100	50	19	2200	53	19	0	0	-	-	-	-	-	-
2050	3 2 1	-	-	-	27	1927	32	-	-	-	14	0	23	29	1405	60	13	0	26
2386	3 1 2	28	220	57	28	0	57	-	-	-	6	0	42	-	-	-	-	-	-
2637	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	29	1120	66	10	<1	36
2670	1 3 2	20	440	27	20	440	2.7	0	0	0	0	0	27	-	-	-	-	-	-
2704	3 1 2	-	-	-	29	2100	52	-	-	-	15	0	50	29	1652	89	16	<1	41
2745	1 3 2	10	190	47	10	190	47	10	0	0	10	0	0	-	-	-	-	-	-
3055	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3159	3 1 2	-	-	-	16	1400	43	-	-	-	7	0	32	28.8	624	94.5	19.2	<1	38.4
3162	3 2 1	25	1800	58	25	1550	58	-	-	-	8	0	38	31	1553	73	8	0	33
3305	2 1 3	-	-	-	35	2000	55	-	-	-	11	<1	<1	38	1400	70	15	<1	34
3339	1 2 3	18	2100	42	18	2100	42	-	-	-	7	0	25	-	-	-	-	-	-
3475	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3511	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	29	1652	70	15	<1	34
3533	1 3 2	0	70	27	0	70	0	-	-	-	-	-	-	-	-	-	-	-	-
3730	2 1 3	35	2100	50	-	-	-	33	0	41	-	-	-	-	-	-	-	-	-
3868	3 2 1	26	1600	42	26	1300	42	23	0	15	16	0	34	36	1700	53	25	0	24
4015	1 2 3	33	1373	49	33	1373	49	27	491	36	23	<1	38	36	1400	56	14	<1	39
4064	1 3 2	20	1650	67	20	1100	67	-	-	-	3	<1	28	-	-	-	-	-	-
4180	3 2 1	-	-	-	20	1100	23	-	-	-	7	0	23	-	-	-	-	-	-
4288	2 1 3	11	1010	49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	1 2 3	31	3350	45	1	2334	45	31	0	42	1	0	0	24	1357	80	13	0	37
4339	2 1 3	-	-	-	35	1400	56	28	0	37	12	0	41	35	1730	56	12	0	37
4343	1 2 3	14	1910	47	14	1676	47	-	-	-	9	0	33	11	1300	74	4	0	39
4356	1 2 3	33	1700	44	33	1700	44	26	0	28	15	0	0	26	2000	48	11	0	28
4539	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	34	1652	70	14	0	38
4633	2 3 1	-	-	-	17	1550	46	19	0	29	5	0	32	27	1596	52	11	0	25
4723	3 1 2	39	1364	44	39	1364	44	-	-	-	16	0	32	-	-	-	-	-	-
4889	2 3 1	-	-	-	32	1700	73	-	-	-	0	0	37	37	1700	74	17	0	34
4980	2 1 3	24	2300	40	24	2300	40	6	0	27	6	0	27	42.9	1676	69.7	22.2	<1	38.4
5018	3 1 2	23	1710	36	23	<1	<1	-	-	-	19	<1	<1	30	1414	63	20	<1	3
5094	3 2 1	400	142	62	400	142	45	21	0	33	0	0	33	-	-	-	-	-	-
5120	1 3 2	42	2600	59	42	2600	59	-	-	-	14	0	24	59	2573	152	35	0	74
5201	2 1 3	27	1450	41	27	1450	0	-	-	-	11	0	0	-	-	-	-	-	-
5220	3 2 1	-	-	-	-	-	-	-	-	-	23	0	25	-	-	-	-	-	-
5352	2 1 3	34	1750	49	34	1550	30	16	0	0	16	0	0	-	-	-	-	-	-
5447	2 3 1	27	200	54	27	200	54	-	-	-	15	0	35	-	-	-	-	-	-
5553	2 3 1	-	-	-	28	1230	35	-	-	-	12	<1,0	35	-	-	-	-	-	-
5893	2 1 3	-	-	-	-	-	-	-	-	-	<1	<1	32	-	-	-	-	-	-
5950	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6180	1 3 2	31	2350	70	31	1900	70	30	0	48	13	0	30	36	1730	62	19	<1	32
6253	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	33	1400	58	13	0	34
6456	1 2 3	-	-	-	33	1450	56	-	-	-	7	0	31	36	1625	52	13	0	34
6563	3 2 1	30	2382	60	30	1786	60	30	2382	60	15	0	15	25	1733	75	8	0	49
6731	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	>1	>1	>1	>1	>1	>1
6852	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	23	1400	62	13.7	0	27.2
7096	1 2 3	-	-	-	24	1860	50	26	0	31	16	0	34	-	-	-	-	-	-
7191	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7248	1 3 2	57	2458	76	32	1827	57	40	0	33	16	0	74	31.4	0	83.3	14.2	2111	44.8
7302	3 2 1	24	1409	48	24	1409	48	-	-	-	15	<1	38	27	1900	82	15	<1	39
7442	2 3 1	32	1718	65	32	1718	65	-	-	-	16	0	50	25	1810	64	10	0	33
7596	1 2 3	29	2000	45	29	2000	45	12	0	57	12	0	36	18	1733	56	10	0	35
7626	1 2 3	20	1300	36	20	1300	36	26	0	40	10	0	30	28	1600	59	14	0	31
7688	3 1 2	-	-	-	10	1300	12	-	-	-	10	0	12	24	3300	54	11	0	26
7728	1 2 3	-	-	-	14	1448	29	-	-	-	0	0	29	-	-	-	-	-	-
7876	4 3 2	35	1791	33	35	1791	33	18	<1	21	15	<1	25	25	2420	70	12	<1	33
7896	3 1 2	14	210	48	14	210	48	20	190	48	<1	<1	<1	-	-	-	-	-	-
Mean					25	1395	48				12	0	32	28	1653	65	13	0	34
CV (%)					15	25	12				20	-	16	11	14	9	15	-	10

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 (3); also briefly described in the text.

Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (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	
0	1450	0	-	-	-	-	-	-	-	-	-	99	17	57	1131
-	-	-	-	-	-	-	-	-	-	-	-	98	30	62	1132
-	-	-	-	-	-	-	-	-	-	-	-	104	24	47	1149
-	-	-	-	-	-	-	-	-	-	-	-	81	29	84	1237
-	-	-	-	-	-	23	0	0	180	0	0	97	25	62	1254
0	2000	0	0	2000	0	110	0	0	140	0	0	82	23	77	1545
-	-	-	-	-	-	80	0	0	160	0	0	109	23	58	1594
-	-	-	-	-	-	50	0	0	230	0	0	57	12	102	1611
0	1150	0	-	-	-	24	0	0	181	0	4	98	25	52	1753
0	1105	0	-	-	-	20	-	-	160	-	-	100	23	52	1868
0	1200	0	0	1200	0	0	0	0	230	0	0	82	21	55	1970
0	1318	0	-	-	-	27	0	0	177	2	0	100	28	83	2050
0	430	0	0	430	0	-	-	-	-	-	-	80	18	60	2386
-	-	-	<1	1500	<1	-	-	-	-	-	-	102	18	51	2637
0	24	0	0	24	0	-	-	-	-	-	-	80	2	55	2670
-	-	-	0	600	0	-	-	-	-	-	-	99	19	56	2704
-	-	-	-	-	-	-	-	-	-	-	-	101	20	72	2745
-	-	-	-	-	-	-	-	-	-	-	-	110	60	60	3055
-	-	-	0	930	0	-	-	-	-	-	-	122	23	61	3076
0	760	0	-	-	-	40	0	0	190	0	0	83	22	52	3162
<1	1100	<1	<1	1100	<1	-	-	-	-	-	-	100	12	74	3305
18	1000	39	0	1000	0	-	-	-	-	-	-	100	24	77	3339
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3475
-	-	-	-	-	-	-	-	-	-	-	-	96	23	58	3511
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3533
-	-	-	-	-	-	-	-	-	-	-	-	104	17	44	3730
0	1200	0	0	1200	0	100	0	0	110	0	0	85	22	58	3868
<1	1222	<1	-	-	-	32	<1	<1	230	<1	<1	114	16	63	4015
-	-	-	-	-	-	-	-	-	-	-	-	94	10	60	4180
-	-	-	-	-	-	-	-	-	-	-	-	84	8	35	4288
-	-	-	0	600	0	90	0	0	190	0	0	95	37	72	4319
0	937	0	-	-	-	7	0	0	65	0	0	66	22	69	4343
0	1200	0	0	1200	0	-	-	-	-	-	-	92	26	69	4356
0	187	0	0	187	0	-	-	-	-	-	-	107	20	66	4539
-	-	-	-	-	-	-	-	-	-	-	-	95	14	69	4633
0	1182	0	-	-	-	50	0	0	209	0	0	92	26	70	4723
-	-	-	0	470	0	-	-	-	-	-	-	110	22	72	4889
0	680	0	0	680	0	-	-	-	-	-	-	89	12	63	4980
<1	850	<1	<1	850	<1	30	<1	<1	230	<1	18	89	20	55	5018
-	-	-	0	0	0	0	0	0	131	0	4	96	19	76	5094
0	1800	0	0	1800	0	9	0	0	192	0	4	123	28	57	5120
-	-	-	-	-	-	-	-	-	-	-	-	93	16	52	5201
-	-	-	-	-	-	-	-	-	-	-	-	112	12	40	5220
0	1327	0	0	1327	0	18	0	0	150	0	10	72	18	58	5352
-	-	-	-	-	-	45	0	0	155	0	0	110	37	69	5447
-	-	-	<1,0	1120	<1,0	-	-	-	-	-	-	105	53	42	5553
-	-	-	-	-	-	-	-	-	-	-	-	107	18	65	5893
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5950
0	1100	0	0	1100	0	-	-	-	-	-	-	120	32	70	6180
-	-	-	-	-	-	-	-	-	-	-	-	104	30	70	6253
-	-	-	-	-	-	-	-	-	-	-	-	105	17	63	6456
0	1200	0	-	-	-	100	0	1	208	0	0	86	25	67	6563
-	-	-	-	-	-	-	-	-	-	-	-	136	10	53	6731
-	-	-	0	1240	0	-	-	-	-	-	-	88	21	53	7096
0	948	0	-	-	-	76	0	0	175	0	10	108	18	55	7248
<1	840	<1	-	-	-	24	<1	9	155	<1	<1	86	31	65	7302
-	-	-	-	-	-	15	0	0	170	0	110	96	20	51	7442
0	590	0	0	590	0	-	-	-	-	-	-	111	25	57	7596
0	1400	0	0	1400	0	40	0	0	165	0	0	102	21	55	7626
-	-	-	0	1100	0	23	0	0	250	0	4	100	19	69	7688
<1	1382	<1	-	-	-	25	<1	<1	175	<1	<1	100	26	57	7728
<1	470	<1	<1	470	<1	30	<1	<1	69	<1	<1	59	<1	57	7876
0	874	0	0	723	0	39	0	0	178	0	0	97	22	62	7896
-	36	-	-	44	-	36	-	-	10	-	-	6	17	9	Mean CV (%)

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria ("rapid" MPN)			<i>E. coli</i> ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7930	3 1 2	18	2100	35	18	2100	35	-	-	-	9	0	0	22	1380	50	9	0	25
7946	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
7962	3 2 1	26	2200	51	26	720	69	24	110	57	14	0	0	32	1700	64	17	0	25
7968	2 3 1	26	2400	53	26	2400	53	27	950	40	10	0	27	30	1582	62	16	0	33
8068	3 1 2	25	1560	44	25	1430	44	18	500	24	9	0	31	28	1730	47	6	0	28
8255	3 2 1	-	-	-	24	1600	56	36	0	44	10	0	49	34	1730	69	11	0	35
8260	1 2 3	83	<1	63	83	<1	63	28	<1	<1	8	<1	<1	-	-	-	-	-	
8329	3 1 2	15	1445	39	15	1445	39	28	0	46	10	0	26	29	1986	59	16	0	37
8380	1 3 2	28	2700	60	28	410	14	-	-	-	14	<1	<1	29	2400	89	20	<1	51
8435	3 1 2	15125	1370	47	15125	1370	47	20	0	20	10	0	38	-	-	-	-	-	
8569	1 2 3	26	3500	48	26	2000	48	-	-	-	-	-	-	-	-	-	-	-	
8598	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8626	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8628	3 1 2	-	-	29	1700	5	6	0	0	6	0	0	0	-	-	-	-	-	
8663	2 3 1	24	3700	68	24	1900	68	38	0	44	12	0	0	32	2000	79	7	0	48
8742	3 1 2	10	1200	30	-	-	-	-	-	-	<1	<1	11	-	-	-	-	-	
8751	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	36	110	56	15	<1	36
8766	1 2 3	24	1900	40	24	1900	40	22	0	24	13	0	32	23	2420	59	15	0	33
8862	2 1 3	49	3364	66	49	1600	66	-	-	-	25	0	41	29	1893	48	17	0	25
8891	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8898	2 1 3	26	1946	51	26	1649	51	-	-	-	13	0	37	22	1722	92	11	0	40
8955	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
9002	1 3 2	-	-	11	1300	10	-	-	-	10	0	40	-	-	-	-	-	-	
9436	1 2 3	21	691	50	21	691	40	19	<1	23	15	<1	35	26	649	55	16	<1	37
9451	3 1 2	27	2200	60	27	2200	60	19	0	50	10	0	35	-	-	-	-	-	
9465	3 1 2	21	1845	65	21	1518	65	-	-	-	13	455	40	22	1733	61	12	<1	35
9569	2 1 3	25	1380	46	25	1380	46	19	<1	34	10	<1	16	25	1733	48	15	<1	23
9655	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	33	540	49	17	<1,8	22
9736	1 2 3	28	2000	41	28	1550	41	-	-	-	17	0	34	28	1690	68	15	0	41
9899	1 3 2	32	2577	69	32	2000	69	29	1423	44	14	0	37	33	1571	73	13	0	41
9903	2 3 1	37	1683	46	37	1350	46	37	1470	38	13	0	25	-	-	-	-	-	
9956	3 2 1	26	3000	51	26	1800	51	26	0	53	10	0	41	39	1732	72	17	0	36

n	62	62	62	75	75	75	42	42	42	77	77	77	57	57	57	57	58	57
Min	0	0	19	0	0	0	0	0	0	0	0	0	11	0	47	4	0	3
Max	15125	3700	76	15125	2600	73	40	2382	60	25	455	74	59	3300	152	35	2111	74
Median	26	1771	48	26	1550	48	23.5	0	36	12	0	32	29	1700	63.5	13	0	35
Mean				25	1395	48				12	0	32	28	1653	65	13	0	34
CV (%)				15	25	12				20	-	16	11	14	9	15	-	10
False positive				0	0	0				0	1	0	0	0	0	0	1	0
False negative				1	3	3				8	0	16	0	1	0	0	0	0
Outliers, low				1	0	5				0	0	0	0	1	0	0	0	1
Outliers, high				4	0	0				0	0	0	1	0	1	1	0	1
Low limit OK	0	0	19	10	70	23	0	0	0	1	0	11	11	540	47	4	0	22
High limit OK	15125	3700	76	49	2600	73	40	2382	60	25	0	74	43	3300	95	25	0	55

mv (\sqrt{Mean})				5.042	37.353	6.955				3.400	0.000	5.666	5.318	40.651	8.048	3.630	0.000	5.872
s ($CV \cdot mv/100$)				0.765	9.399	0.810				0.679	0.000	0.881	0.574	5.681	0.728	0.547	0.000	0.595
$u_{rel,mv}$ (%) ($100 \cdot s / \sqrt{n_{mv}}$)				1.8	3.0	1.4				2.4		2.0	1.4	1.9	1.2	2.0		1.4
x (\sqrt{Result})																		
z ($ x-mv /s$)																		

Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (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	
0	10	0	0	10	0	-	-	-	-	-	-	107	32	63	7930
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7946
-	-	-	-	-	-	70	0	0	140	0	0	85	31	80	7962
0	1529	0	0	1529	0	65	0	0	195	0	0	105	22	64	7968
0	45	0	0	45	0	60	0	0	190	0	0	101	30	54	8068
-	-	-	-	-	-	10	0	2	170	0	0	100	23	56	8255
<1	1008	<1	<1	1008	<1	-	-	-	-	-	-	89	21	63	8260
0	1027	0	-	-	-	20	0	0	154	0	0	999	25	68	8329
-	-	-	-	-	-	40	<1	<1	170	<1	<1	86	19	71	8380
0	370	0	0	370	0	-	-	-	-	-	-	100	14	66	8435
-	-	-	-	-	-	-	-	-	-	-	-	90	27	85	8569
-	-	-	-	-	-	-	-	-	-	-	-	114	40	78	8598
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8626
-	-	-	0	74	0	120	0	0	150	0	0	161	22	68	8628
0	830	0	0	830	0	-	-	-	-	-	-	95	30	77	8663
-	-	-	-	-	-	-	-	-	-	-	-	100	20	61	8742
-	-	-	-	-	-	-	-	-	-	-	-	93	17	68	8751
0	882	0	-	-	-	23	0	0	140	0	0	95	29	57	8766
0	2200	0	-	-	-	64	0	0	209	0	0	109	25	57	8862
-	-	-	-	-	-	-	-	-	-	-	-	98	19	55	8891
0	1636	0	-	-	-	72	0	0	195	0	0	93	27	62	8898
-	-	-	0	1800	0	32	0	0	210	0	0	77	26	54	8955
<1	891	<1	-	-	-	5	<1	<1	100	<1	<1	87	10	46	9436
0	230	0	0	230	0	-	-	-	-	-	-	96	25	59	9451
-	-	-	-	-	-	-	-	-	-	-	-	104	26	59	9465
<1	1	<1	<1	1	<1	8	<1	<1	190	<1	<1	82	22	75	9569
-	-	-	-	-	-	-	-	-	-	-	-	105	6	57	9655
0	667	0	-	-	-	49	0	0	205	0	0	96	25	57	9736
0	1727	0	-	-	-	54	0	0	200	0	0	104	31	65	9899
0	1231	0	-	-	-	24	0	0	206	0	0	97	23	49	9903
-	-	-	-	-	-	-	-	-	-	-	-	93	37	58	9956

45	45	45	35	35	35	43	42	42	43	42	42	92	92	92	n
0	1	0	0	1	0	0	0	0	65	0	0	11	0	35	Min
18	2200	39	0	2000	0	120	0	9	250	2	110	999	60	102	Max
0	1027	0	0	930	0	32	0	0	180	0	0	98	23	60	Median
0	874	0	0	723	0	39	0	0	178	0	0	97	22	62	Mean
-	36	-	-	44	-	36	-	-	10	-	-	6	17	9	CV (%)
1	0	1	0	0	0	0	0	3	0	1	8	0	0	0	False pos.
0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	False neg.
0	0	0	0	0	0	0	0	0	2	0	0	3	1	0	Outliers <
0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	Outliers >
0	1	0	0	1	0	5	0	0	100	0	0	66	6	35	Low limit
0	2200	0	0	2000	0	120	0	0	250	0	0	136	53	102	High limit

0.000	29.564	0.000	0.000	26.888	0.000	6.242	0.000	0.000	13.345	0.000	0.000	9.843	4.699	7.843	mv
0.000	10.666	0.000	0.000	11.771	0.000	2.273	0.000	0.000	1.274	0.000	0.000	0.595	0.781	0.677	s
5.4			7.4			5.7			1.5			0.6	1.8	0.9	$u_{rel,mv}$ (%)
															x
															z

Annex B Z-scores calculated from the laboratory results. Susp. = Suspected on the membrane filters before confirmation. $z = (x - \bar{m}) / s$. Z-scores are calculated also for outliers (excluding false negative results) in the same way as ordinary z-scores. From false positive results can no z-scores be calculated. Z-scores from outliers are not real z-scores

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
1131	1 2 3				0.569	0.021	-0.033				0.094	0.000	-0.871	-0.047	0.172	0.681	-0.572	0.000	0.354
1132	1 3 2										0.094	0.000*		-0.382	1.504	-0.066	-0.572	0.000	0.215
1149	2 3 1																		
1237	3 2 1				4.000	0.413	-0.305				0.000	-0.215		-1.671	-0.809	0.019	-0.572	0.000	0.893
1254	2 1 3				0.804	0.282	0.144				0.094	0.000	-0.756						
1545	3 2 1				-1.046	-2.684	-2.172				-1.401	0.000	-1.230						
1594	1 3 2				-1.046	1.238	-0.122				0.094	0.000	1.350	-1.474	0.312	-0.868	-0.303	0.000	-0.070
1611	2 3 1				0.687	0.147	-0.397				0.697	0.000	0.747	-1.474	-0.537	-0.239	-0.572	0.000	-1.301
1753	1 3 2				0.449	0.431	-0.876				-0.123	0.000	-0.534	-0.213	0.639	-0.504	-0.572	0.000	0.215
1868	3 2 1				0.074	-0.138	0.570				-0.590	0.000	1.267	-0.382	0.012	1.459	-0.854	0.000	2.597
1970	1 2 3				0.804	-0.445	0.144				1.413	0.000							
2050	3 2 1				0.201	0.696	-1.603				0.503	0.000	-0.988	0.116	-0.558	-0.415	-0.044	0.000	-1.301
2386	3 1 2				0.326	0.735					-1.401	0.000	0.925						
2637	3 1 2													0.116	-1.265	0.104	-0.854	0.000	0.215
2670	1 3 2				-0.746	-1.742	4.000				0.000	-0.534							
2704	3 1 2				0.449	0.902	0.317				0.697	0.000	1.595	0.116	-0.001	1.903	0.677	0.000	0.893
2745	1 3 2				-2.459	-2.508	-0.122				-0.350	0.000							
3055	2 3 1																		
3076	1 2 3																		
3159	3 1 2				-1.363	0.007	-0.491				-1.112	0.000	-0.011	0.084	-2.758	2.297	1.374	0.000	0.546
3162	3 2 1				-0.055	0.215	0.816				-0.842	0.000	0.566	0.434	-0.219	0.681	-1.464	0.000	-0.215
3305	2 1 3				1.143	0.784	0.570				-0.123	0.000		1.474	-0.569	0.437	0.444	0.000	-0.070
3339	1 2 3				-1.046	0.902	-0.585				-1.112	0.000	-0.756						
3475	1 3 2													0.116	-0.001	0.437	0.444	0.000	-0.070
3511	1 3 2																		
3533	1 3 2																		
3730	2 1 3																		
3868	3 2 1				0.074	-0.138	-0.585				0.884	0.000	0.187	1.187	0.102	-1.055	2.504	0.000	-1.637
4015	1 2 3				0.919	-0.032	0.056				2.057	0.000	0.566	1.187	-0.569	-0.776	0.204	0.000	0.627
4064	1 3 2				-0.746	-0.445	1.520				-2.458	0.000	-0.425						
4180	3 2 1				-0.746	-0.445	-2.666				-1.112	0.000	-0.988						
4288	2 1 3																		
4319	1 2 3				4.000	1.166	-0.305				-3.537	0.000		-0.731	-0.671	1.230	-0.044	0.000	0.354
4339	2 1 3				1.143	0.007	0.653				0.094	0.000	0.837	1.041	0.166	-0.776	-0.303	0.000	0.354
4343	1 2 3				-1.701	0.382	-0.122				-0.590	0.000	0.089	-3.487	-0.809	0.761	-2.978	0.000	0.627
4356	1 2 3				0.919	0.413	-0.397				0.697	0.000		-0.382	0.116	-1.538	-0.572	0.000	-0.977
4539	1 2 3													0.893	-0.001	0.437	0.204	0.000	0.491
4633	2 3 1				-1.202	0.215	-0.213				-1.715	0.000	-0.011	-0.213	-0.123	-1.149	-0.572	0.000	-1.468
4723	3 1 2				1.573	-0.045	-0.397				0.884	0.000	-0.011						
4889	2 3 1				0.804	0.413	1.962							1.332	0.102	0.761	0.901	0.000	-0.070
4980	2 1 3				-0.187	1.128	-0.778				-1.401	0.000	-0.534	2.145	0.051	0.413	1.977	0.000	0.546
5018	3 1 2				-0.322						1.413	0.000		0.277	-0.537	-0.152	1.539	0.000	4.000
5094	3 2 1				4.000	-2.706	-0.305							0.000	0.089				
5120	1 3 2				1.882	1.451	0.897				0.503	0.000	-0.871	4.000	1.773	4.000	4.000	0.000	4.000
5201	2 1 3				0.201	0.077					0.123	0.000							
5220	3 2 1													2.057	0.000	-0.756			
5352	2 1 3				1.032	0.215	-1.825				0.884	0.000							
5447	2 3 1				0.201	-2.470	0.486				0.697	0.000	0.284						
5553	2 3 1				0.326	-0.243	-1.283				0.094	0.000	0.284						
5893	2 1 3										0.000	-0.011							
5950	2 1 3																		
6180	1 3 2				0.687	0.664	1.743				0.303	0.000	-0.215	1.187	0.166	-0.239	1.332	0.000	-0.363
6253	2 1 3										0.742	-0.569	-0.594	-0.044	0.000	-0.070			
6456	1 2 3				0.919	0.077	0.653				-1.112	0.000	-0.112	1.187	-0.060	-1.149	-0.044	0.000	-0.070
6563	3 2 1				0.569	0.522	0.977				0.697	0.000	-2.035	-0.555	0.172	0.840	-1.464	0.000	1.897
6731	1 3 2													0.884	0.000	0.187	-0.910	-0.569	-0.239
6852	2 1 3				-0.187	0.614	0.144							0.131	0.000	-1.105			
7096	1 2 3																		
7191	2 1 3																		
7248	1 3 2				0.804	0.574	0.735				0.884	0.000	3.333	0.497	1.481	0.253	1.381		
7302	3 2 1				-0.187	0.020	-0.033				0.697	0.000	0.566	-0.213	0.517	1.383	0.444	0.000	0.627
7442	2 3 1				0.804	0.436	1.368				0.884	0.000	1.595	-0.555	0.333	-0.066	-0.854	0.000	-0.215
7596	1 2 3				0.449	0.784	-0.305				0.094	0.000	0.379	-1.874	0.172	-0.776	-0.854	0.000	0.073
7626	1 2 3				-0.746	-0.138	-1.179				-0.350	0.000	-0.215	-0.047	-0.115	-0.504	0.204	0.000	-0.513
7688	3 1 2				-2.459	-0.138	-4.000				-0.350	0.000	-2.500	-0.731	2.956	-0.961	-0.572	0.000	-1.301
7728	1 2 3				-1.701	0.074	-1.938				0.697	0.000	-0.319						
7876	1 3 2				1.143	0.529	-1.494				0.697	0.000	-0.756	-0.555	1.504	0.437	-0.303	0.000	-0.215
7896	3 1 2				-1.701	-2.432	-0.033				0.000								
7930	3 1 2				-1.046	0.902	-1.283				-0.590	0.000		-1.094	-0.617	-1.342	-1.151	0.000	-1.468
7946	2 1 3																		
7962	3 2 1				0.074	-1.119	1.669				0.503	0.000		0.590	0.102	-0.066	0.901	0.000	-1.468
7968	2 3 1				0.074	1.238	0.402				-0.350	0.000	-0.534	0.277	-0.154	-0.239	0.677	0.000	-0.215
8068	3 1 2				-0.055	0.049	-0.397				-0.590	0.000							

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)			Total plate count 22 °C, 3 days			Lab no.		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
0.000	0.798	0.000					*		0.179	-0.738	-0.433	1131					
									0.094	0.997	0.046	1132					
									0.596	0.256	-1.459	1149					
									-1.417	0.879	1.953	1237					
									0.009	0.385	0.046	1254					
0.000	1.421	0.000	0.000	1.515	0.000	-0.636	0.000	0.000	0.056	0.000	0.000	1.324	0.124	1.377	1545		
						1.868	0.000	0.000	-1.188	0.000	0.000	1.003	0.124	-0.336	1594		
						1.189	0.000	0.000	-0.546	0.000	0.000	-3.854	-1.582	3.334	1611		
						0.365	0.000	0.000	1.429	0.000	0.000	0.094	0.385	-0.934	1753		
						-0.591	0.000	0.000	0.085	0.000	-0.546	0.263	0.124	-0.934	1868		
						-0.778						-1.324	-0.150	-0.631	1970		
									0.263	0.759	1.873	2050					
									-0.460	0.000	0.000	-0.032	0.000	-1.511	-0.585	-0.144	2386
												0.430	-0.585	-1.037	2637		
												-1.511	-4.000	-0.631	2670		
												0.179	-0.436	-0.532	2704		
												0.347	-0.291	0.949	2745		
												1.083	3.903	-0.144	3055		
												2.020	0.124	-0.049	3076		
												-4.000	0.256	-0.144	3159		
												-0.76	0.124	-0.336	3162		
												0.263	-1.582	1.122	3305		
												0.263	0.256	1.377	3339		
												-0.076	0.124	-0.336	3475		
												0.596	-0.738	-1.788	3511		
												-1.048	-0.011	-0.336	3533		
												1.401	-0.896	0.139	3730		
												-0.249	-1.969	-0.144	3868		
												-1.140	-2.397	-2.847	4015		
												-0.423	0.124	1.207	4064		
												-0.162	1.773	0.949	4180		
												-2.889	-0.011	0.685	4339		
												-0.423	0.512	0.685	4343		
												0.841	-0.291	0.415	4356		
												-0.162	-1.227	0.685	4539		
												-0.423	0.512	0.774	4633		
												0.365	0.000	0.000	4723		
												0.872	0.000	0.000	4889		
												-0.336	-0.688	-1.139	4980		
												-0.336	-0.688	-0.631	5018		
												-0.076	-0.436	1.292	5094		
												2.096	0.759	-0.433	5120		
												-0.336	-0.896	-0.934	5201		
												1.243	-1.582	-2.244	5220		
												-2.282	-0.585	-0.336	5352		
												1.083	1.773	0.685	5447		
												0.678	3.307	-2.013	5553		
												0.841	-0.585	0.324	5893		
												1.867	1.227	0.774	5950		
												0.596	0.997	0.774	6180		
												0.678	-0.738	0.139	6253		
												-0.957	0.385	0.506	6456		
												3.056	-1.969	-0.832	6563		
												-0.777	-0.150	-0.832	6731		
															6852		
															7096		
															7191		
												1.089	0.000	0.000	7248		
												-0.092	0.000	0.000	7302		
												-0.703	0.000	0.000	7442		
												-0.241	0.000	0.000	7596		
												1.163	0.385	-0.433	7626		
												0.430	-0.150	-0.631	7688		
												0.263	-0.436	0.685	7728		
												-0.891	0.256	-1.037	7876		
												0.263	0.512	-0.433	7896		
												-0.336	-3.633	-0.433	7930		
												0.841	1.227	0.139	7946		
												0.935	0.000	0.000	7962		
												-1.188	0.000	0.000	7968		
												0.801	0.000	0.000	8068		
												0.486	0.000	0.000	8255		
												0.662	0.000	0.000	8260		
												-1.355	0.000	-0.241	8329		
												0.734	0.000	0.000	8380		
												0.037	0.000	0.000	8435		
												-0.241	0.000	0.000	8598		
												0.935	-0.599	0.637	8626		
												4.000	-0.011	0.596	8663		
												0.000	-1.554	0.000			
												2.073	0.000	0.000			
												-0.862	0.000	0.000			
												4.000	-0.162	0.997			
												0.000	0.163	0.000			

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
8742	3 1 2										0.000	-2.667							
8751	1 3 2										1.187	-4.000	-0.776	0.444	0.000	0.215			
8766	1 2 3				-0.187	0.664	-0.778				0.303	0.000	-0.011	-0.910	1.504	-0.504	0.444	0.000	-0.215
8862	2 1 3				2.561	0.282	1.444				2.358	0.000	0.837	0.116	0.503	-1.538	0.901	0.000	-1.468
8891	2 3 1																		
8898	2 1 3				0.074	0.346	0.231				0.303	0.000	0.473	-1.094	0.149	2.119	-0.572	0.000	0.761
8955	3 2 1																		
9002	1 3 2				-2.257	-0.138	-4.000				-0.350	0.000	0.747						
9436	1 2 3				-0.601	-1.177	-0.778				0.697	0.000	0.284	-0.382	-2.671	-0.868	0.677	0.000	0.354
9451	3 1 2				0.201	1.016	0.977				-0.350	0.000	0.284						
9465	3 1 2				-0.601	0.171	1.368				0.303	0.000	0.747	-1.094	0.172	-0.327	-0.303	0.000	0.073
9569	2 1 3				-0.055	-0.022	-0.213				-0.350	0.000	-1.891	-0.555	0.172	-1.538	0.444	0.000	-1.811
9655	3 1 2													0.742	-3.065	-1.439	0.901	0.000	-1.988
9736	1 2 3				0.326	0.215	-0.681				1.065	0.000	0.187	-0.047	0.081	0.272	0.444	0.000	0.893
9899	1 3 2				0.804	0.784	1.669				0.503	0.000	0.473	0.742	-0.179	0.681	-0.044	0.000	0.893
9903	2 3 1				1.361	-0.065	-0.213				0.303	0.000	-0.756						
9956	3 2 1				0.074	0.540	0.231				-0.350	0.000	0.837	1.614	0.170	0.600	0.901	0.000	0.215
n		0	0	0	74	72	72	0	0	0	69	76	61	57	56	57	57	57	57
Min					-4.000	-3.084	-4.000				-3.537	0.000	-2.667	-3.487	-4.000	-1.638	-2.978	0.000	-4.000
Max					4.000	1.451	1.962				2.358	0.000	3.333	4.000	2.956	4.000	4.000	0.000	4.000
Median																			
Mean					0.138	0.215	-0.122				0.094	0.000	-0.011	0.116	0.091	-0.066	-0.044	0.000	0.073
SD					0.162	0.000	-0.277				0.000	0.000	0.000	0.070	-0.071	0.070	0.070	0.000	0.000
z<-3					1.414	1.000	1.405				1.000	0.000	1.000	1.124	1.126	1.124	1.124	0.000	1.239
-3≤z<-2																			
2<=z≤3																			
z>3																			
					1	1	5				1	0	0	1	2	0	0	0	1
					3	5	2				1	0	3	0	2	0	2	0	0
					1	0	0				3	0	0	1	1	2	1	0	2
					4	0	0				0	0	1	1	0	1	1	0	1

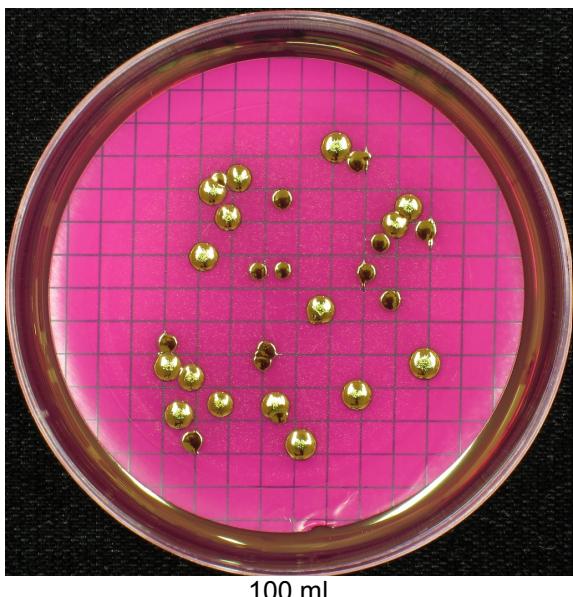
Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Total plate count 22 °C, 3 days			Lab no.	
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
0.000	0.013	0.000				-0.636	0.000	0.000	-1.188	0.000	0.000	0.263	-0.291	-0.049	8742	
0.000	1.626	0.000				0.774	0.000	0.000	0.872	0.000	0.000	-0.336	-0.738	0.596	8751	
0.000	1.021	0.000				0.987	0.000	0.000	0.486	0.000	0.000	-0.162	0.879	-0.433	8766	
			0.000	1.320	0.000	-0.257	0.000	0.000	0.899	0.000	0.000	1.003	0.385	-0.433	8862	
						-1.762	0.000	0.000	-2.625	0.000	0.000	-0.336	0.637	0.046	8891	
0.000	0.027	0.000							-1.795	0.512	-0.731	-0.423	-0.738	-1.141	8955	
0.000	-1.350	0.000								-0.867	-1.969	-1.567	-0.076	0.385	-0.239	9002
0.000	-2.678	0.000								-0.423	-1.141	9436	0.596	0.512	-0.239	9451
			0.000	-2.199	0.000	-1.501	0.000	0.000	0.344	0.000	0.000	-1.324	-0.011	1.207	9465	
0.000	-0.350	0.000				0.334	0.000	0.000	0.763	0.000	0.000	-0.076	0.385	-0.433	9569	
0.000	1.125	0.000				0.487	0.000	0.000	0.625	0.000	0.000	0.596	1.113	0.324	9655	
0.000	0.518	0.000				-0.591	0.000	0.000	0.791	0.000	0.000	0.009	0.124	-1.246	9736	
												-0.336	1.773	-0.336	9899	
															9903	
															9956	
44	45	44	35	35	35	41	42	39	43	41	34	92	91	92	n	
0.000	-2.678	0.000	0.000	-2.199	0.000	-1.762	0.000	0.000	-4.000	0.000	0.000	-4.000	-4.000	-2.847	Min	
0.000	1.626	0.000	0.000	1.515	0.000	2.073	0.000	0.000	1.935	0.000	0.000	4.000	3.903	3.334	Max	
0.000	0.233	0.000	0.000	0.307	0.000	-0.257	0.000	0.000	-0.032	0.000	0.000	0.052	0.124	-0.144	Median	
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.185	0.000	0.000	-0.038	-0.001	0.000	Mean	
0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.293	0.000	0.000	1.334	1.151	1.000	SD	
0	0	0	0	0	0	0	0	0	2	0	0	3	1	0	Summa	
0	4	0	0	2	0	0	0	0	2	0	0	2	2	3	18	
0	0	0	0	0	0	1	0	0	0	0	0	2	1	1	33	
0	0	0	0	0	0	0	0	0	0	0	0	3	2	1	16	
															15	

Annex C – photos

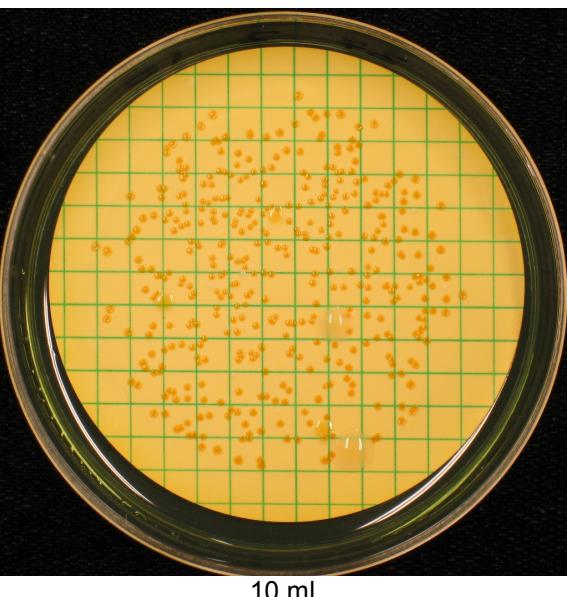
Drinking water, March 2013

Mixture A

m-Endo Agar LEE, 37 °C



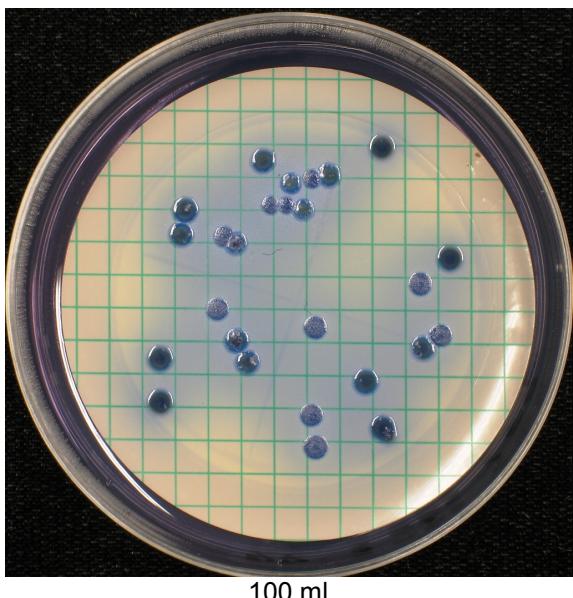
100 ml



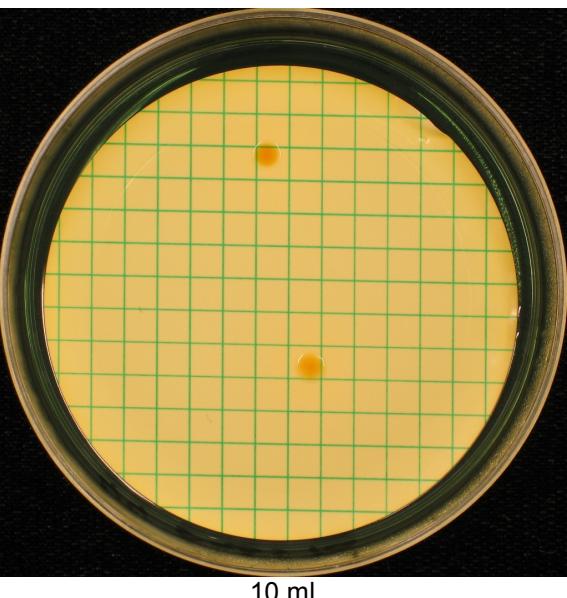
10 ml

m-Lactose TTC Agar, 37 °C

m-FCC Agar, 44 °C



100 ml

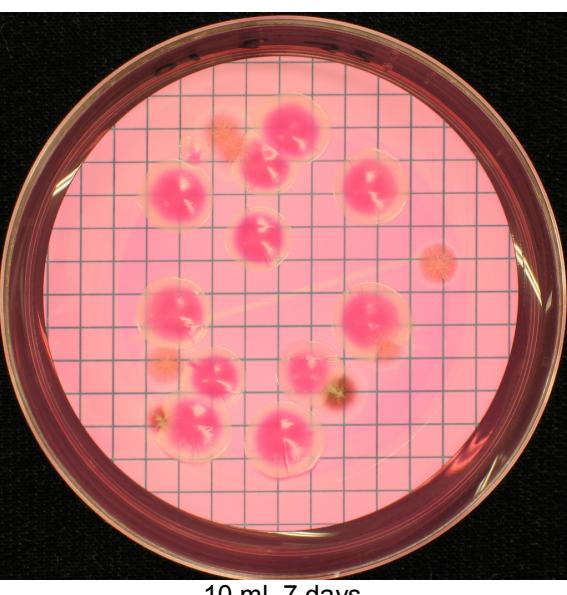


10 ml

m-Lactose TTC Agar, 44 °C

TSC Agar, 44 °C

10 ml



10 ml, 7 days

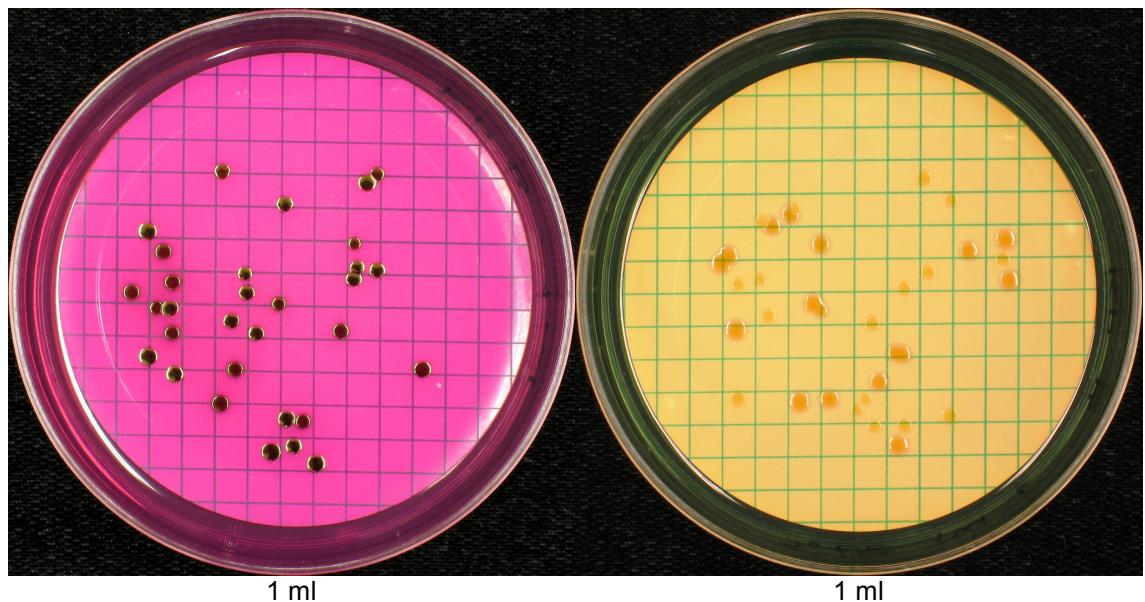
TSC Agar, 44 °C

m-Lactose TTC Agar, 37 °C

m-Lactose TTC Agar, 44 °C

RBCC Agar, 25 °C

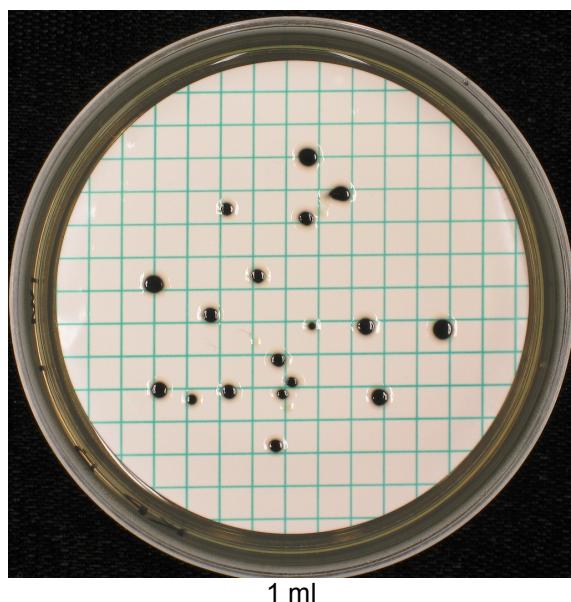
Mixture B



m-Endo Agar L:ES, 37 °C

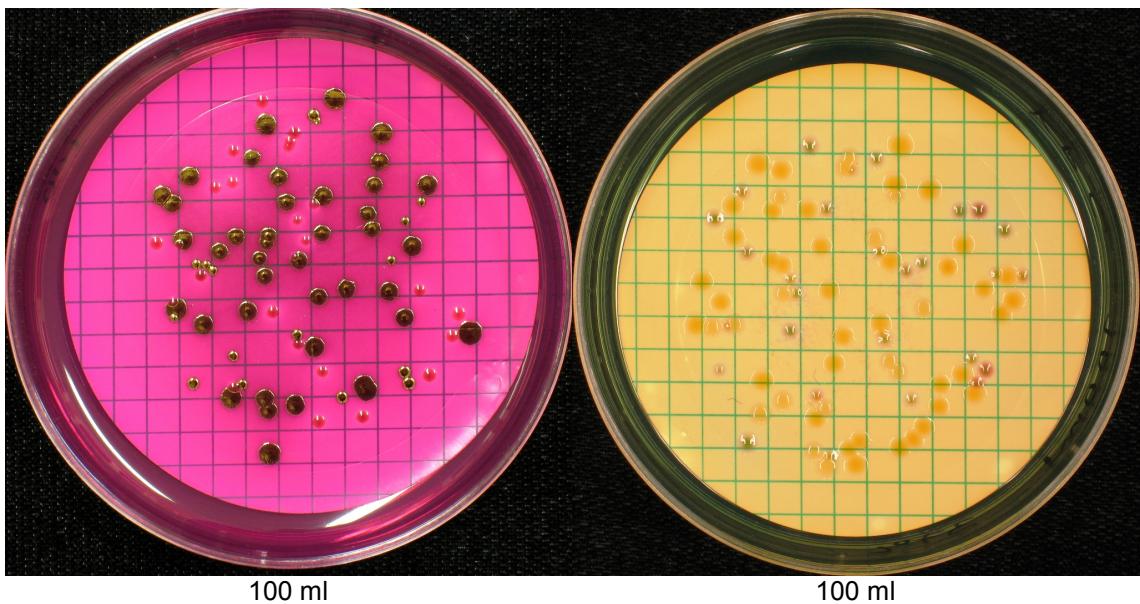
m-FC Agar, 44 °C

TSC Agar, 44 °C



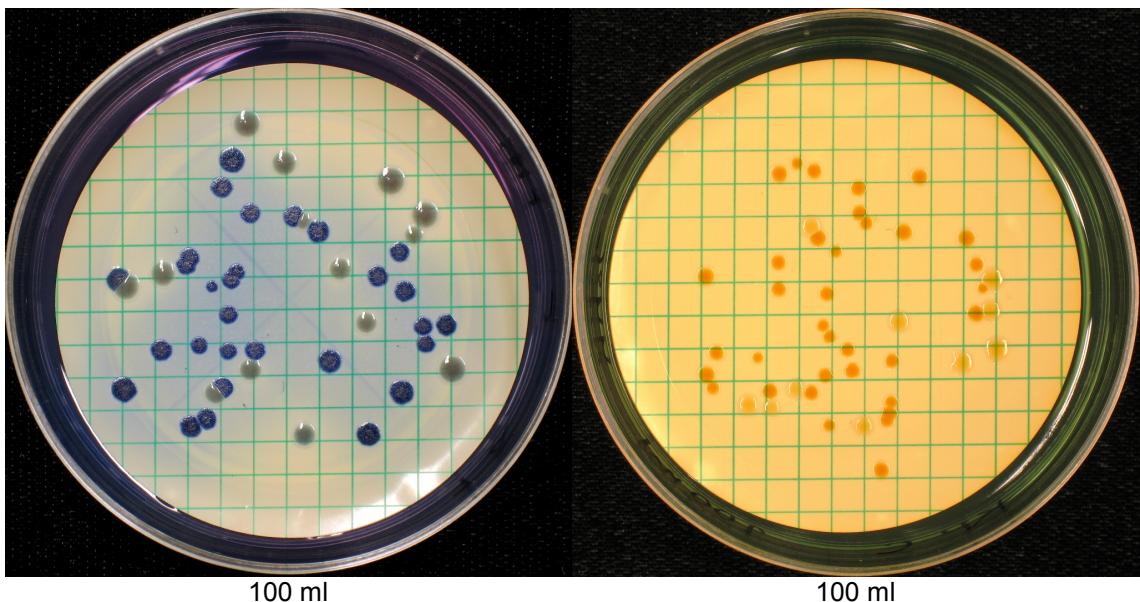
Mixture C

m-Endo Agar L/E/S, 37 °C



m-Lactose TTC Agar, 37 °C

m-FCC Agar, 44 °C



m-Lactose TTC Agar, 44 °C

TSC Agar, 44 °C

RBCC Agar, 25 °C

1. Fisk, skaldjur och fiskprodukter – analys av näringssämnen av V Öhrvik, A von Malmborg, I Mattisson, S Wretling och C Åstrand.
2. Normerande kontroll av dricksvattenanläggningar 2007-2010 av T Lindberg.
3. Tidstrender av tungmetaller och organiska klorerade miljöföroringar i baslivsmedel av J Ålander, I Nilsson, B Sundström, L Jorhem, I Nordlander, M Aune, L Larsson, J Kuivinen, A Bergh, M Isaksson och A Glynn.
4. Proficiency Testing – Food Microbiology, January 2012 by C Normark, I Boriak and L Nachin.
5. Mögel och mögelgifter i torkad frukt av E Fredlund och J Spång.
6. Mikrobiologiska dricksvattenrisker ur ett kretsloppsperspektiv – behov och åtgärder av R Dryselius.
7. Market Basket 2010 – chemical analysis, exposure estimation and health-related assessment of nutrients and toxic compounds in Swedish food baskets.
8. Proficiency Testing – Food Microbiology, April 2012 by L Nachin, C Normark, I Boriak and I Tillander.
9. Kontroll av restsubstanser i levande djur och animaliska livsmedel. Resultat 2010 av I Nordlander, Å Kjellgren, A Glynn, B Aspenström-Fagerlund, K Granelli, I Nilsson, C Sjölund Livsmedelsverket och K Girma, Jordbruksverket.
10. Råd om fullkorn 2009 - bakgrund och vetenskapligt underlag av W Becker, L Busk, I Mattisson och S Sand.
11. Nordiskt kontrollprojekt 2012. Märkning av allergener och ”kan innehålla spår av allergener” – resultat av de svenska kontrollerna av U Fäger.
12. Proficiency Testing – Drinking Water Microbiology, 2012:1, March by T Šlapokas, M Lindqvist and K Mykkänen.
13. Länsstyrelsens rapportering av livsmedelskontroll inom primärproduktionen 2010-2011 av L Eskilsson och K Bäcklund Stålenheim.
14. Vetenskapligt underlag för råd om mängden frukt och grönsaker till vuxna och barn av H Eneroth.
15. Kommuner och Livsmedelsverkets rapportering av livsmedelskontrollen 2011 av L Eskilsson.
16. Sammanställning av resultat från en projektinriktad kontrollkurs om skyddade beteckningar 2012 av P Elvingsson.
17. Nordic Expert Survey on Future Foodborne and Waterborne Outbreaks by T Andersson, Å Fulke, S Pesonen and J Schlundt.
18. Riksprojekt 2011. Kontroll av märkning – redlighet och säkerhet av C Spens, U Colberg, A Göransdotter Nilsson och P Bergkvist.
19. Från nutritionsforskning till kostråd – så arbetar Livsmedelsverket av I Mattisson, H Eneroth och W Becker.
20. Proficiency Testing – Food Microbiology, October 2012 by L Nachin ,C Normark and I Boriak
21. Dioxin- och PCB-halter i fisk och andra livsmedel 2000-2011 av T Cantillana och M Aune.
22. Not publiced.
23. Kontroll av kontaminanter i livsmedel 2011 – Resultat från kontrollprogrammen för dioxiner och dioxinlika PCB, PAH, nitrat, mykotoxiner och tungmetaller av A Wannberg, F Broman och H Omberg.
24. Proficiency Testing – Drinking Water Microbiology, 2012:2, September by T Šlapokas and K Mykkänen.

1. Contaminants and minerals in foods for infants and young children – analytical results, Part 1, by V Öhrvik, J Engman, B Kollander and B Sundström.
Contaminants and minerals in foods for infants and young children – risk and benefit assessment, Part 2 by G Concha, H Enerothe, H Hallström and S Sand.
Tungmetaller och mineraler i livsmedel för spädbarn och småbarn. Del 3 Risk- och nyttohantering av R Bjerselius, E Halldin Ankarberg, A Jansson, I Lindeberg, J Sanner Färnstrand och C Wanhainen.
Contaminants and minerals in foods for infants and young children – risk and benefit management, Part 3 by R Bjerselius, E Halldin Ankarberg, A Jansson, I Lindeberg, J Sanner Färnstrand and C Wanhainen.
2. Bedömning och dokumentation av näringriktiga skolluncher – hanteringsrapport av A-K Quetel.
3. Gluten i maltdrycker av Y Sjögren och M Hallgren.
4. Kontroll av bekämpningsmedelsrester i livsmedel 2010 av A Wannberg, A Jansson och B-G Ericsson.
5. Proficiency Testing – Food Microbiology, January 2013 by L Nachin ,C Normark and I Boriak.
6. Från jord till bord – risk- och sårbarhetsanalys. Rapport från nationellt seminarium i Stockholm november 2012.
7. Cryptosporidium i dricksvatten – riskvärdering av R Lundqvist, M Egervärn och T Lindberg.
8. Proficiency Testing – Food Microbiology, April 2013 av L Nachin, C Normark, I Boriak and I Tillander.
9. Proficiency Testing – Drinking Water Microbiology, March 2013 by T Šlapokas and K Mykkänen.