

Drinking Water Microbiology

March 2014

by Tommy Šlapokas and Kirsi Mykkänen



Edition

Version 1 (2014-06-04)

Editor in chief

Hans Lindmark, Head of Microbiology Division, National Food Agency

Responsible for the scheme

Tommy Šlapokas, Microbiologist, Microbiology Division, National Food Agency

PT March 2014 is registered as no. 780/2014 at the National Food Agency, Uppsala

Proficiency testing
Drinking water Microbiology
March 2014



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

Moulds with MF

Yeasts with MF

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

Tommy Šlapokas & Kirsi Mykkänen

Abbreviations and explanations

Microbiological media

LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (acc. to EN-ISO 9308-: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; ISO/DIS 9308-1:2013)
Colilert	Colilert [®] Quanti-Tray [®] (IDEXX Inc.; ISO 9308-2:2012)

Other abbreviations

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

Legend to method comparison tables

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

Contents

Abbreviations and explanations	2
General information on results evaluation	4
Results of the PT round March 2014	4
- General outcome	4
- Coliform bacteria (MF)	6
- Suspected thermotolerant coliform bacteria (MF)	8
- <i>Escherichia coli</i> (MF)	10
- Coliform bacteria and <i>E. coli</i> (rapid method, MPN)	12
- Presumptive and confirmed <i>Clostridium perfringens</i> (MF)	14
- Moulds and yeasts (MF)	17
- Culturable microorganisms 22 °C, 3 days	20
Outcome of the results and laboratory assessment	22
- Assessment of the performance	22
- General information about reported results	22
- Mixed up samples or results	22
- Z-scores, box plots and deviating results for each laboratory	22
Test material, quality control and processing of data	26
- Description of the test material	26
- Quality control of the test material	26
- Processing of numerical results	27
References	29
Annex A – All reported results	30
Annex B – Z-scores of the results	34
Annex C – Photo example of colony appearance on some media	38

General information on results evaluation

The histograms and calculation of outliers are described on page 27 under "Processing of numerical results" with further reference to the scheme protocol (1).

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

The method information gathered is sometimes difficult to interpret. Sometimes there is no consistency between the standard referred to and the information given regarding various method details. Results from laboratories with ambiguous details are either excluded or placed in the group "Other/Unknown" in the tables, together with results from methods used only by individual laboratories.

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in tables together with the group means etc. The measure of dispersion is not shown for groups with 4 or fewer results.

Results of the PT round March 2014

General outcome

Test items were sent to 98 laboratories, 37 in Sweden, 49 in other Nordic countries (Faeroe Islands and Åland included), 3 more from EU, 2 from the rest of Europe and 7 from countries outside Europe. Results were reported from 97 laboratories.

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

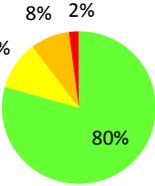
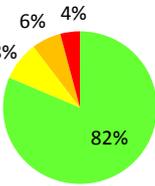
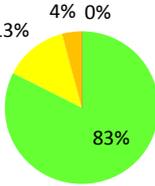
Microorganisms and parameters of analyses are also compiled in **table 1**. For the MF analyses the parameters *suspected* coliform bacteria and thermotolerant coliform bacteria on primary media could be reported as well. The results from suspected colonies are only used for interpretations and discussions.

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

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

Laboratory performance is described on page 22, after all analytical parameters, and illustrated by a box plot together with the number of deviating results for each laboratory.

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

Mixture	A			B			C		
Percentage of laboratories with									
No. of evaluable results	531			529			535		
No. of deviating results *	34 (6 %)			33 (6 %)			21 (4 %)		
Microorganisms	<i>Klebsiella pneumoniae</i> <i>Hafnia alvei</i> <i>Clostridium perfringens</i> <i>Acremonium strictum</i>			<i>Escherichia coli</i> <i>Aeromonas hydrophila</i> <i>Phialophora fastigiata</i> <i>Issatchenkia orientalis</i> <i>Staphylococcus cohnii</i>			<i>Klebsiella pneumoniae</i> <i>Klebsiella oxytoca</i> <i>Clostridium bifermentans</i> <i>Candida glabrata</i>		
Analysis	Target	F%	X%	Target	F%	X%	Target	F%	X%
Coliform bacteria (MF)	<i>K. pneumoniae</i> { <i>H. alvei</i> }	1	3	<i>E. coli</i> [<i>A. hydrophila</i>]	1	8	<i>K. pneumoniae</i> <i>K. oxytoca</i>	0	3
Susp. thermotolerant colif. bact. (MF)	<i>K. pneumoniae</i>	–	–	<i>E. coli</i>	–	–	<i>K. pneumoniae</i>	–	–
<i>E. coli</i> (MF)	[<i>K. pneumoniae</i>]	5	–	<i>E. coli</i>	3	3	[<i>K. pneumoniae</i>]	9	–
Coliform bacteria (rapid method)	<i>K. pneumoniae</i> { <i>H. alvei</i> }	0	0	<i>E. coli</i>	0	0	<i>K. pneumoniae</i> <i>K. oxytoca</i>	0	0
<i>E. coli</i> (rapid meth.)	–	0	–	<i>E. coli</i>	0	0	–	0	–
Presumptive <i>C. perfringens</i> (MF)	<i>C. perfringens</i>	2	0	–	0	–	<i>C. bifermentans</i>	0	2
<i>Clostridium perfringens</i> (MF)	<i>C. perfringens</i>	5	0	–	3	–	[<i>C. bifermentans</i>]	10	–
Moulds (MF)	<i>A. strictum</i>	24	5	<i>Ph. fastigiata</i>	5	17	–	2	–
Yeasts (MF)	–	23	–	<i>I. orientalis</i>	17	0	<i>C. glabrata</i>	2	7
Culturable micro-organisms (total count), 3 days 22 °C	<i>H. alvei</i> <i>K. pneumoniae</i>	0	3	<i>S. cohnii</i> (<i>E. coli</i>) (<i>A. hydrophila</i>)	1	3	{ <i>C. glabrata</i> } <i>K. pneumoniae</i> <i>K. oxytoca</i>	0	2

* In total 40 of 97 laboratories (41%) reported at least one deviating result

– Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

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

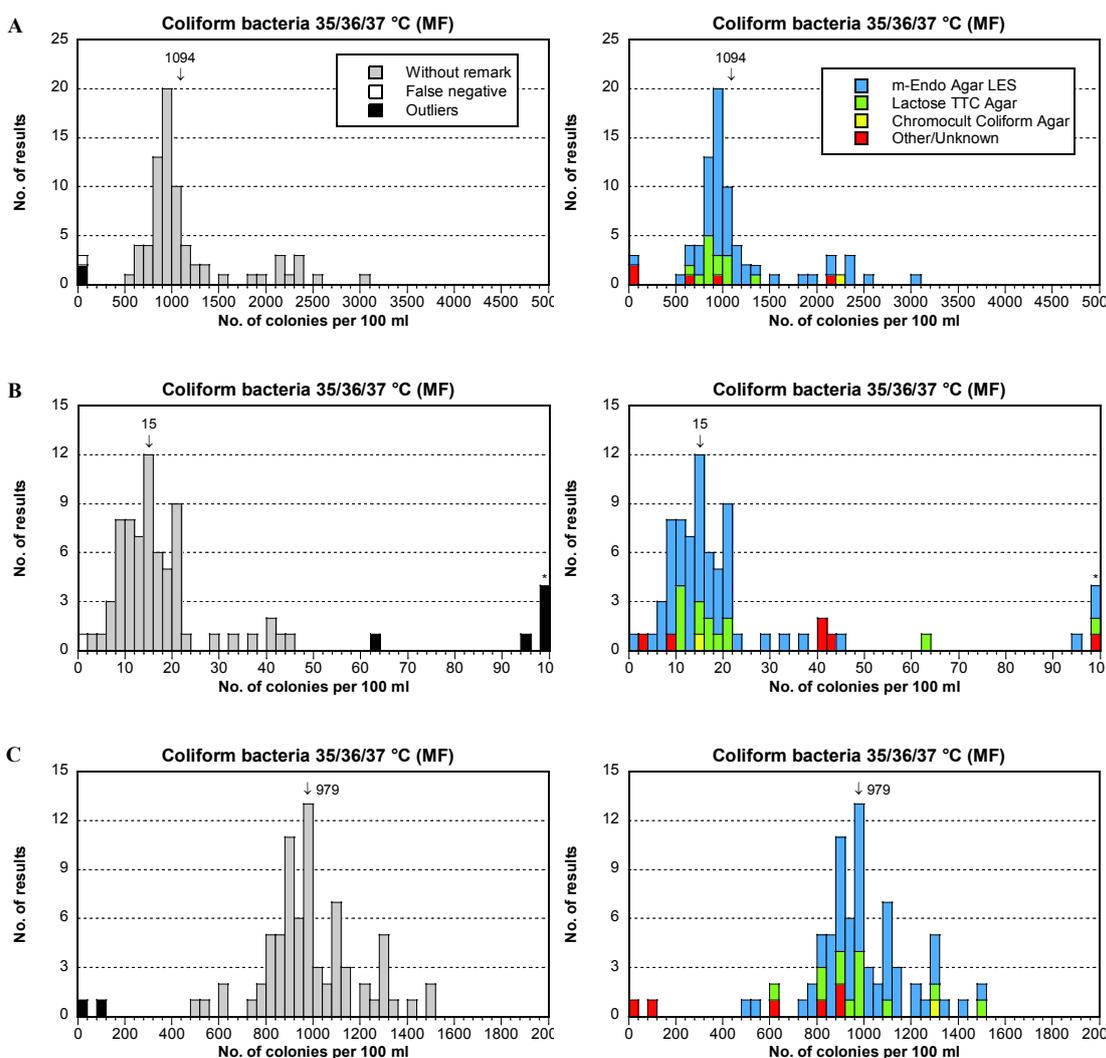
{ } The organism may give different results depending on method or definition used

Coliform bacteria (MF)

In 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 LES was four times more frequently used than LTTC. There is an indication that LTTC gave a somewhat lower mean result compared to

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	76	72	1094	20	1	2	0	68	15	26	1	0	6	74	979	10	0	2	0
m-Endo Agar LES	56	55	1121	21	1	0	0	51	15	23	1	0	3	56	990	10	0	0	0
Lactose TTC Agar	13	13	909	8	0	0	0	11	14	14	0	0	2	13	968	11	0	0	0
Chromocult C Agar	1	1	2200	–	0	0	0	1	14	–	0	0	0	1	1300	–	0	0	0
Other/Unknown	6	3	1149	–	0	2	0	4	31	–	0	0	1	4	787	–	0	2	0



LES, in particular in mixture A. However, the dispersion and thus the uncertainty are lower for LTTC in both mixture A and B. As seen from the histograms and the table about 1/3 of the results in all mixtures are low for the category Other/Unknown, in several cases the results are even outliers.

Mixture A

- *K. pneumoniae* form colonies in the analysis of coliform bacteria and accounts for the largest peak in the histogram. The colonies are typical with a metallic sheen on LES and pale yellow on LTTC at 37 °C.
- In the minor peak with higher results, colonies of *H. alvei* have also been included as coliform bacteria, which could be correct. However, their colonies are often red without metallic sheen on LES and greenish on LTTC, and are as such usually excluded as suspected coliform bacteria. But apparently, these colonies are sometimes included on LES – but not LTTC – which indicate that they sometimes get a metallic sheen on LES.

Mixture B

- The colonies of both *E. coli* and *A. hydrophila* are typical for suspected coliform bacteria on both LES and LTTC, but with somewhat different appearance. Individual yellow colour beneath the colonies in LTTC is, as usual, difficult to discern as the whole plate often turns yellow.
- *A. hydrophila* is oxidase positive and might have less metallic sheen than *E. coli*, and accordingly more red colonies. The colonies can with certainty be excluded from coliform bacteria after confirmation by the oxidase test.
- The number of *A. hydrophila* was about 10 times higher than *E. coli*. This explains the high outliers where all or at least a part of the *A. hydrophila* colonies must have been counted as coliform bacteria. Probably also the same explanation is valid for the other results above 30 cfu/100 ml, since such results are absent from the rapid method.

Mixture C

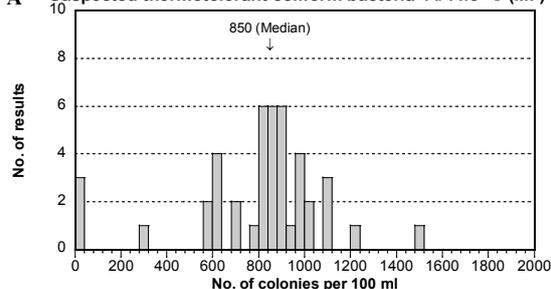
- Colonies of both *K. pneumoniae* and *K. oxytoca* grow with typical appearance on the media for coliform bacteria. Negative oxidase test confirms that they are coliform bacteria.
- The histogram seems to have more than one peak. However, this is probable illusionary, because also the corresponding histogram for the rapid method has the same range, but with less obvious "separate peaks".
- The two low outliers have been obtained by methods in the category Other/Unknown. One was with m-Lauryl Sulfate Agar by "Standard methods 9221" (5) and the other was with LES in conjunction with the standard EN ISO 9308-1:2000, which is not a correct alternative.

Suspected thermotolerant coliform bacteria (MF)

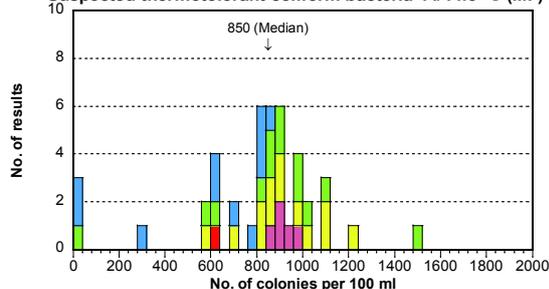
The two most used growth media were m-FC and LTTC. The incubation temperature was 44 or 44.5 °C. Here, results were separated based on the method standards most commonly used, to get a further division. They are EN ISO 9308-1 (2000) with LTTC and three standards with m-FC from the Nordic countries, namely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

Standard, Method	Tot n	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
Total	45	43	850	-	-	-	44	11	-	-	-	44	424	-	-	-
EN ISO 9308-1	11	11	710	-	-	-	11	12	-	-	-	11	450	-	-	-
SS 028167	13	13	890	-	-	-	13	11	-	-	-	13	418	-	-	-
SFS 4088	13	13	885	-	-	-	13	11	-	-	-	13	460	-	-	-
NS 4792	6	5	890	-	-	-	5	8	-	-	-	5	360	-	-	-
Other/Unknown	2	1	620	-	-	-	2	8	-	-	-	2	275	-	-	-

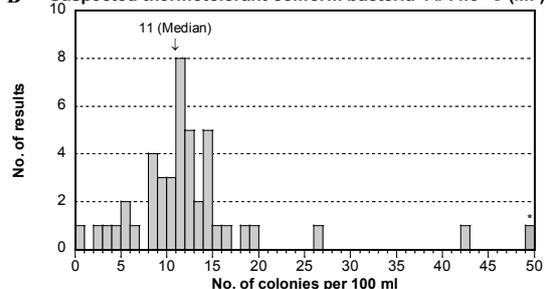
A Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



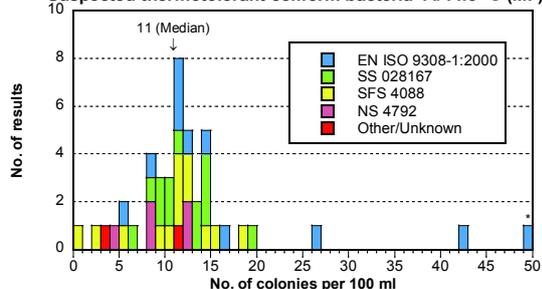
Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



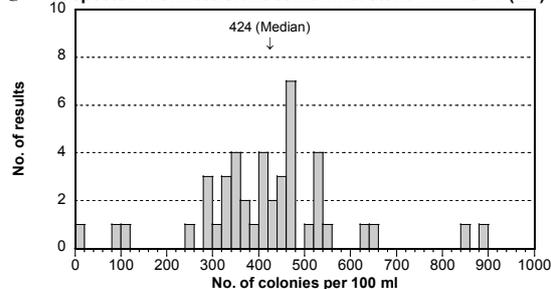
B Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



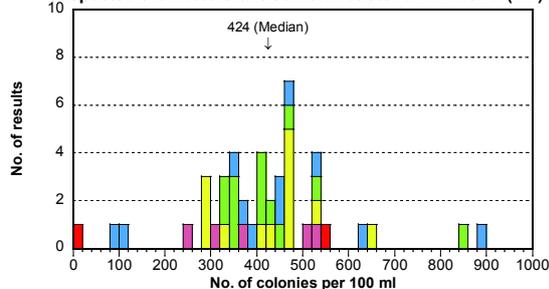
Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



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



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



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

The Swedish standard states incubation at 44 °C but one laboratory used 44.5 °C. The temperature 44 °C is also stated in EN ISO 9308-1:2000. Two laboratories using Norwegian standard have incubated at 44 °C while the other five have used 44.5 °C. All laboratories using Finnish standard incubated at 44 °C.

In both mixture B and C, a somewhat lower median was obtained by the laboratories using Norwegian standard, NS 4792, compared to most other groups. Probably, it is caused by the fact that 5 of the laboratories incubated at 44.5 °C, since the median for the 7 laboratories incubating at 44.5 °C was 8 cfu/100 ml in mixture B and 360 cfu/100 ml in mixture C. For unknown reason, laboratories using LTTC at 44 °C with EN ISO 9308-1:2000 obtained lower results with mixture A than laboratories using m-FC with Swedish, Finnish or Norwegian standard.

Mixture A

- Only colonies of *K. pneumoniae* appear with blue colonies on m-FC at 44/44.5 °C. The corresponding colonies are orange-yellow on LTTC.
- Based on the lower results, it appears that it is more difficult to judge the strain of *K. pneumoniae* as (suspected) thermotolerant coliform bacteria from LTTC than from m-FC.

Mixture B

- The strain of *E. coli* grows as (suspected) thermotolerant coliform bacteria on both m-FC and LTTC. It seems to grow somewhat poorer at 44.5 compared to 44 °C.
- The reason for the tail with low results is unclear, as it is for the highest results.

Mixture C

- The same strain of *K. pneumoniae* as in mixture A grows at 44/44.5 °C. The strain of *K. oxytoca* does not appear.
- The results by Norwegian standard are lower than the other in this mixture, which is different from the results for the same strain in mixture A. The reason for this difference is not clear.

Escherichia coli (MF)

E. coli is quantified after confirmation of colonies that have grown either at 36±2 °C or at 44/44.5 °C. The primary growth media LTTC or LES are used at 36 °C and LTTC or m-FC at 44/44.5 °C. The results from the two temperatures are here shown in separate tables. The 21 results with unclear incubation temperature are not separately shown but are included only in the table "All results".

E. coli was present only in mixture B. No difference between methods was seen at 36±2 or at 44/44.5 °C.

In mixture A and C were 4 and 7 false positive results present, respectively. This indicates that confirmation tests were not performed or performed insufficiently.

Mixture A

- No *E. coli* was included in the mixture. The strain of *K. pneumoniae* grows at both 36±2 and 44/44.5 °C, but is excluded as presumptive *E. coli* due to its lack of indol production and β-glucuronidase activity.
- The 4 false positive results are explained by no or insufficient confirmation.

All results

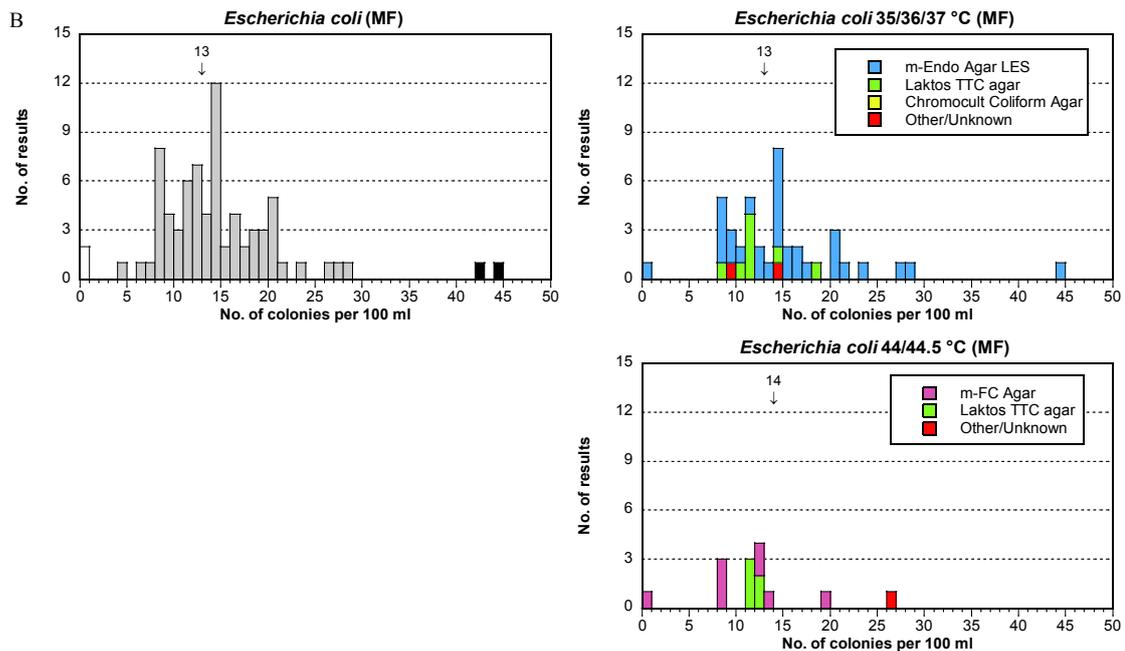
Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	76	71	0	-	4	-	-	71	13	18	2	0	2	68	0	-	7	-	-

From 36±2 °C

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	41	41	0	-	0	-	-	39	14	18	1	-	1	36	0	-	5	-	-
m-Endo Agar LES	31	31	0	-	0	-	-	29	14	18	1	-	1	27	0	-	4	-	-
Lactose TTC Agar	8	8	0	-	0	-	-	8	12	12	0	-	-	7	0	-	1	-	-
Chromocult C Agar	0	0	-	-	-	-	-	0	0	-	-	-	-	0	-	-	-	-	-
Other/Unknown	2	2	0	-	0	-	-	2	11	-	0	-	-	2	0	-	0	-	-

From 44/44.5 °C

Medium/Standard	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	14	13	0	-	1	-	-	13	12	18	1	-	-	13	0	-	1	-	-
<i>Medium</i>																			
m-FC Agar	8	7	0	-	1	-	-	7	11	17	1	-	-	7	0	-	1	-	-
Lactose TTC Agar	5	5	0	-	0	-	-	5	11	2	0	-	-	5	0	-	0	-	-
Other/Unknown	1	1	0	-	0	-	-	1	26	-	-	-	-	1	0	-	0	-	-
<i>Standard</i>																			
EN ISO 9308-1	7	7	0	-	0	-	-	7	13	20	0	-	-	7	0	-	0	-	-
SS 028167	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
SFS 4088	1	1	0	-	0	-	-	1	19	-	0	-	-	1	0	-	0	-	-
NS 4792	4	4	0	-	0	-	-	4	10	-	0	-	-	4	0	-	0	-	-
Other/Unknown	2	1	0	-	1	-	-	1	13	-	1	-	-	1	0	-	1	-	-



Mixture B

- There was one strain of *E. coli* present in the mixture as the only coliform bacterium. Confirmation is needed when *E. coli* is quantified from the primary analysis at 36 ± 2 °C to discriminate it from *A. hydrophila*. Confirmation by indol production or β -glucuronidase activity is further needed to state the presence of *E. coli*.
- Badly performed or excluded confirmation is the probable cause of the 2 high outliers. Also the false negative result from 36 ± 2 °C can be due to insufficient confirmation in the way that all tested colonies were *A. hydrophila*.

Mixture C

- No *E. coli* was included in the mixture but the same strain of *K. pneumoniae* as in mixture A was included together with a strain of *K. oxytoca* that is indole positive. Both strains grow as typical coliform bacteria. Therefore, confirmation is necessary, both with primary growth at 36 ± 2 and $44/44.5$ °C, to ascertain that no *E. coli* is present.
- Colonies of *K. oxytoca* grown at $36 \pm$ °C may produce indole at 44 °C in confirmation broths containing tryptophane. However, the strain will not produce gas at that temperature and has no β -glucuronidase activity.
- *K. pneumoniae* grows at $44/44.5$ °C but lacks indol production and β -glucuronidase activity.
- The 7 false positive results are explained by no or insufficient confirmation.

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. One laboratory has not used a rapid method but the classical multiple tube method with MPN quantification (Standard Methods 9221B; 5). For *E. coli* one laboratory used a qualitative method, "Colilert Presence/Absence". Out of the ca 60 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 wells (a few of which, probably incorrectly, have reported 96 wells). The laboratories often analysed both diluted and undiluted samples. The two laboratories included in the category Other/Unknown stated the use of "Colilert 24 hours".

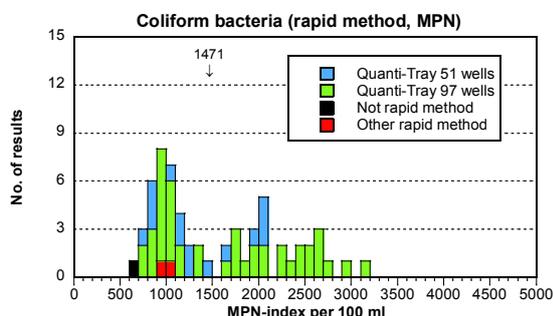
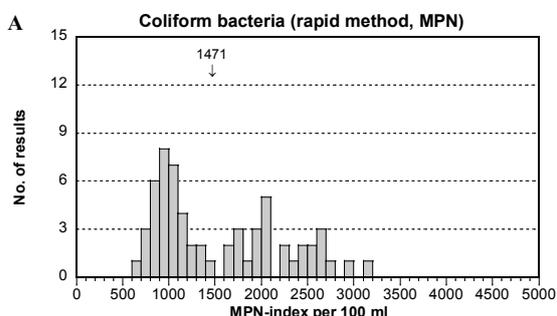
For coliform bacteria in mixture A and C it seems that the trays with 51 wells give somewhat lower average recovery than trays with 97 wells. No deviating results were discerned.

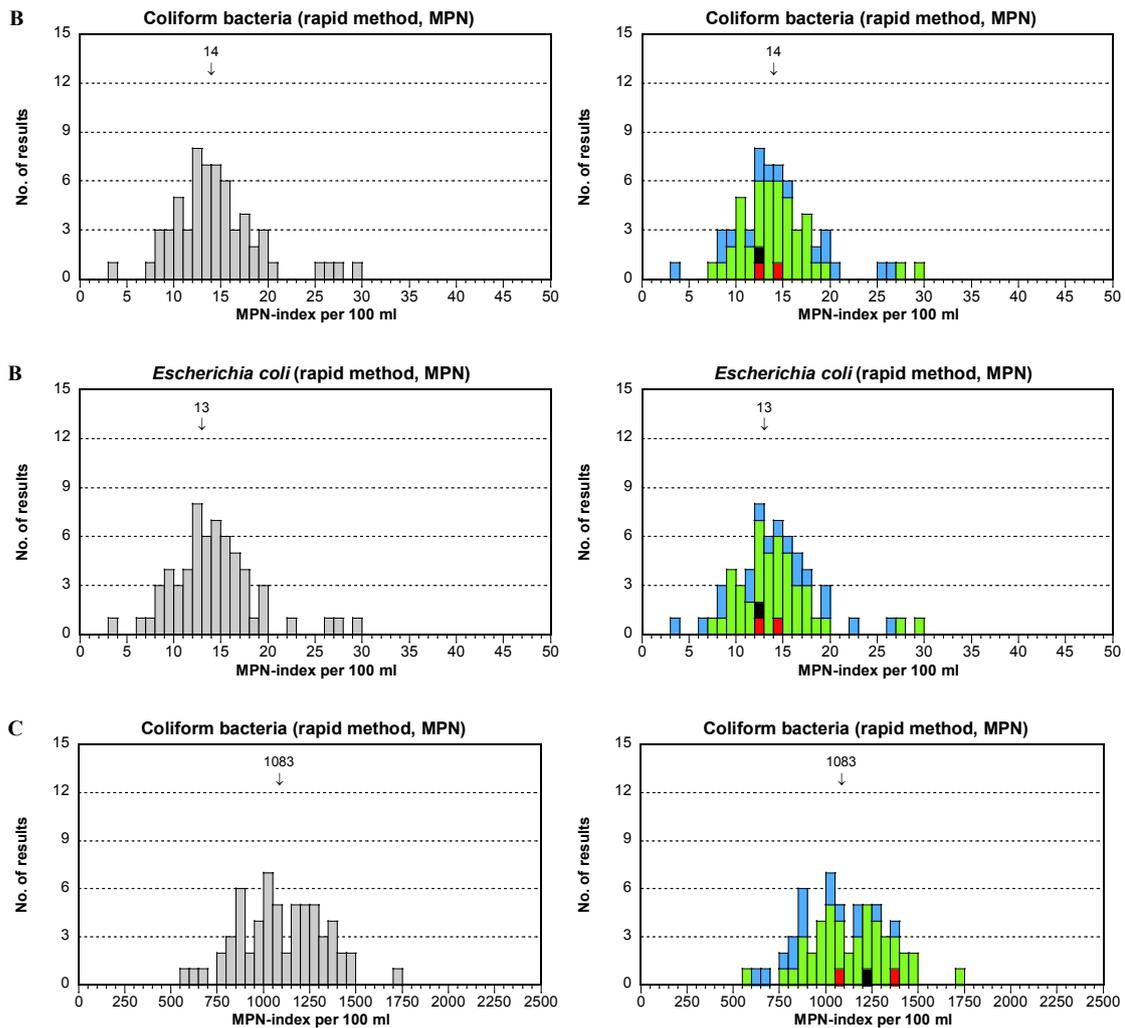
Coliform bacteria, Rapid method with MPN

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	61	60	1487	21	0	0	0	60	14	17	0	0	0	60	1081	11	0	0	0
Colilert Quanti-51	16	15	1328	17	0	0	0	16	14	24	0	0	0	15	956	11	0	0	0
Colilert Quanti-97	43	43	1568	22	0	0	0	42	14	15	0	0	0	43	1119	10	0	0	0
Colilert Quanti-?	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Other/Unknown	2	2	1031	-	0	0	0	2	13	-	0	0	0	2	1231	-	0	0	0
Not rapid method	1	1	690	-	0	0	0	1	12	-	0	0	0	1	1200	-	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.	61	60	0	-	0	-	-	60	13	17	0	0	0	60	0	-	0	-	-
Colilert Quanti-51	17	17	0	-	0	-	-	17	13	23	0	0	0	17	0	-	0	-	-
Colilert Quanti-97	42	41	0	-	0	-	-	41	13	15	0	0	0	41	0	-	0	-	-
Colilert Quanti-?	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
Other/Unknown	2	2	0	-	0	-	-	2	13	-	0	0	0	2	0	-	0	-	-
Not rapid method	2	2	0	-	0	-	-	1	12	-	0	0	0	1	0	-	0	-	-





Mixture A

- *K. pneumoniae* is a typical coliform bacterium, 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.
- The strain of *H. alvei* is an atypical coliform bacterium and is only weakly ONPG positive. After 18 hours of incubation, the result for this strain is negative but turns usually positive after 22 hours of incubation. This fact explains the different peaks and the scattered appearance of results in the histogram, as well as the much higher average cfu per 100 ml (1471) compared to the MF method (1094). The leftmost peak corresponds to detection of only *K. pneumoniae* and the rightmost the sum of this strain and *H. alvei*. The peak in between is somewhat intermediate. Out of the 60 laboratories 36 have stated the incubation time to 18 hours and 11 to 19-20 hours, probably irrespectively if they perform a later 2nd reading or not. If so, the reported incubation time gives no clue to when the detection of *H. alvei* is positive.
- No strain has the enzyme β -glucuronidase and is detected as *E. coli*.

Mixture B

- The *E. coli* strain was the only one detected as a coliform bacterium, based on the activity of the enzyme β -galactosidase, as well as an *E. coli*. The growth of *A. hydrophila* was inhibited by antibiotics in the medium. The average result was approximately the same as for the MF-method.
- Both for coliform bacteria as well as *E. coli* there are 4 results that are higher than the rest. The cause is not clear but is probably not explained by individual cases with *A. hydrophila* being positive. Instead it is probable that individual vials contained somewhat higher numbers of *E. coli* than what was supposed. Even though the mixture was homogenous, two such results (23 and 28 cfu per 100 ml) were available among 20 results in our original homogeneity check.

Mixture C

- Both *K. pneumoniae* and *K. oxytoca* 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.
- The histogram shows the sum of both strains and was without peculiarities. The average result for coliform bacteria was slightly higher than with the MF-method.
- No *E. coli* was present in the mixture and no false positive result was reported.

Presumptive and confirmed *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 rounds. The ISO version of the standard is available since November 2013. It has obtained the designation ISO 14189 (2013) and is basically equivalent to the CD version from 2002 after adjustments but has a much more simplified confirmation step. In the new standard, isolated colonies are only tested for activity of the enzyme acid phosphatase. When the new standard has been included in regulations and guidance documents, the CD version will be invalid to use.

Some other methods/media were used by individual participants, such as NMKL 95:5 (2009) as well as EN ISO 26461-2:1993 modified. The latter contains the same

base medium (PAB/TSC) as in ISO/CD 6461-2 and ISO 14189 but without any confirmation step, and is basically intended for analysis of spores only.

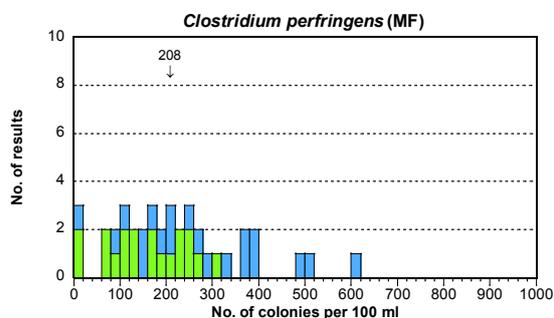
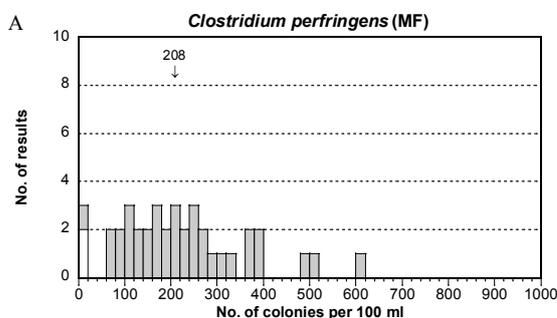
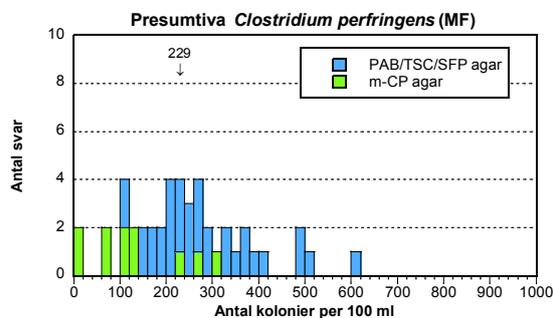
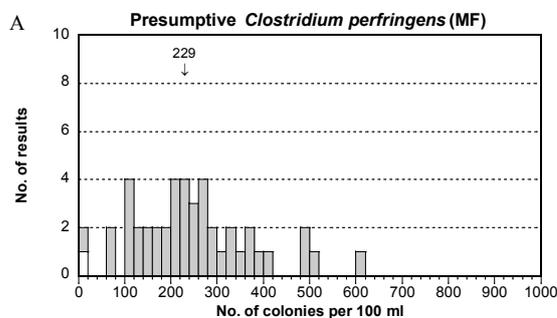
Using m-CP Agar yielded lower numbers for both presumptive *C. perfringens* and *C. perfringens* in mixture A than using PAB/TSC/SFP Agar. The histograms show clearly that m-CP Agar has mainly yielded among the lower part of the results. The same scenario is also seen for presumptive *C. perfringens* in mixture C, where the false positive *C. bifermentans* was present. Only three results higher than zero were reported for m-CP Agar. The CV was very high for both media in mixture C.

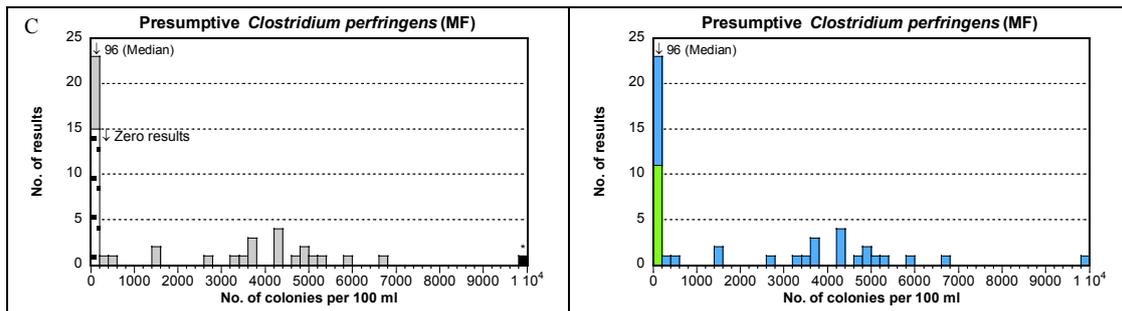
Presumptive Clostridium perfringens MF

Medium	Tot n	A					B					C							
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	45	44	229	28	1	0	0	42	0	-	1	-	-	44	861	105	0	0	1
PAB/TSC/SFP agar	34	34	267	21	0	0	0	33	0	-	0	-	33	1469	80	0	0	1	
m-CP agar	11	10	123	38	1	0	0	9	0	-	1	-	11	6	174	0	0	0	
Iron Sulfite agar	0	0	-	-	-	-	-	0	-	-	-	-	0	-	-	-	-	-	

Clostridium perfringens MF

Medium	Tot n	A					B					C						
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<
Total	40	37	208	31	2	0	0	38	0	-	1	-	-	36	0	-	4	-
PAB/TSC/SFP agar	20	19	272	26	1	0	0	20	0	-	0	-	16	0	-	4	-	
m-CP agar	20	18	150	29	1	0	0	18	0	-	1	-	20	0	-	0	-	
Iron Sulfite agar	0	0	-	-	-	-	-	0	-	-	-	-	0	-	-	-	-	





Mixture A

- A strain of *C. perfringens* was present. Different results were obtained depending on the method used according to the tables and figures.
- The colour of the colonies with PAB/TSC/SFP may vary from light grey-brown to completely black, at least partly depending on the condition of the medium and its reduction potential.
- The distribution of the results is as usual very wide and atypical compared to other parameters. This makes it almost impossible to identify outliers.
- In comparison with PAB/TSC/SFP, most results obtained with m-CP Agar are subjectively considered as deviating low for the strain included.

Mixture B

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

Mixture C

- A strain of *C. bifermentans* was included as a false positive for *C. perfringens*.
- In the presumptive analysis 15 zero results were present, while most other results were more normally distributed.
- There were 4 false positive results for *C. perfringens*, of which 3 also were reported as false positive presumptive colonies. The confirmation seems in those cases to have been incorrectly performed.

Moulds and yeasts (MF)

Out of the 42 laboratories that analysed moulds and yeasts, 32 reported the use of the Swedish standard SS 028192. Besides Sweden it is used in Denmark and also in Finland and Norway under their own national designations SFS 5507 and NS 4716, respectively. Two Finnish laboratories used "Malt Extract Agar" (MEA) in conjunction with SFS 5507. Ten laboratories used other methods, such as Glucose Yeast Extract agar with oxytetracycline as inhibiting substance (OGYE) according to the standard ISO 7954 (1987), DRBC (see below) connected to NMKL 98, modified, or MEA without connection to any standard. Also Dichloran Glycerol 18% (DG 18) as well as Sabouraud-agar was used by one laboratory each. Standard Methods of Water and Wastewater (5) with DRBC was used by one laboratory.

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 agar base", "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 is reported as used, which usually is in accordance with the recommendations from the manufacturer 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 are in many cases unclear. The Swedish laboratories are usually using chlortetracycline and chloramphenicol together in the Rose Bengal agar, as stated in the standard SS 028192. This medium is here designated RBCC agar from the English spelling of the ingredients. Another used name is "Rose Bengal agar according to Burman" based on its promoter.

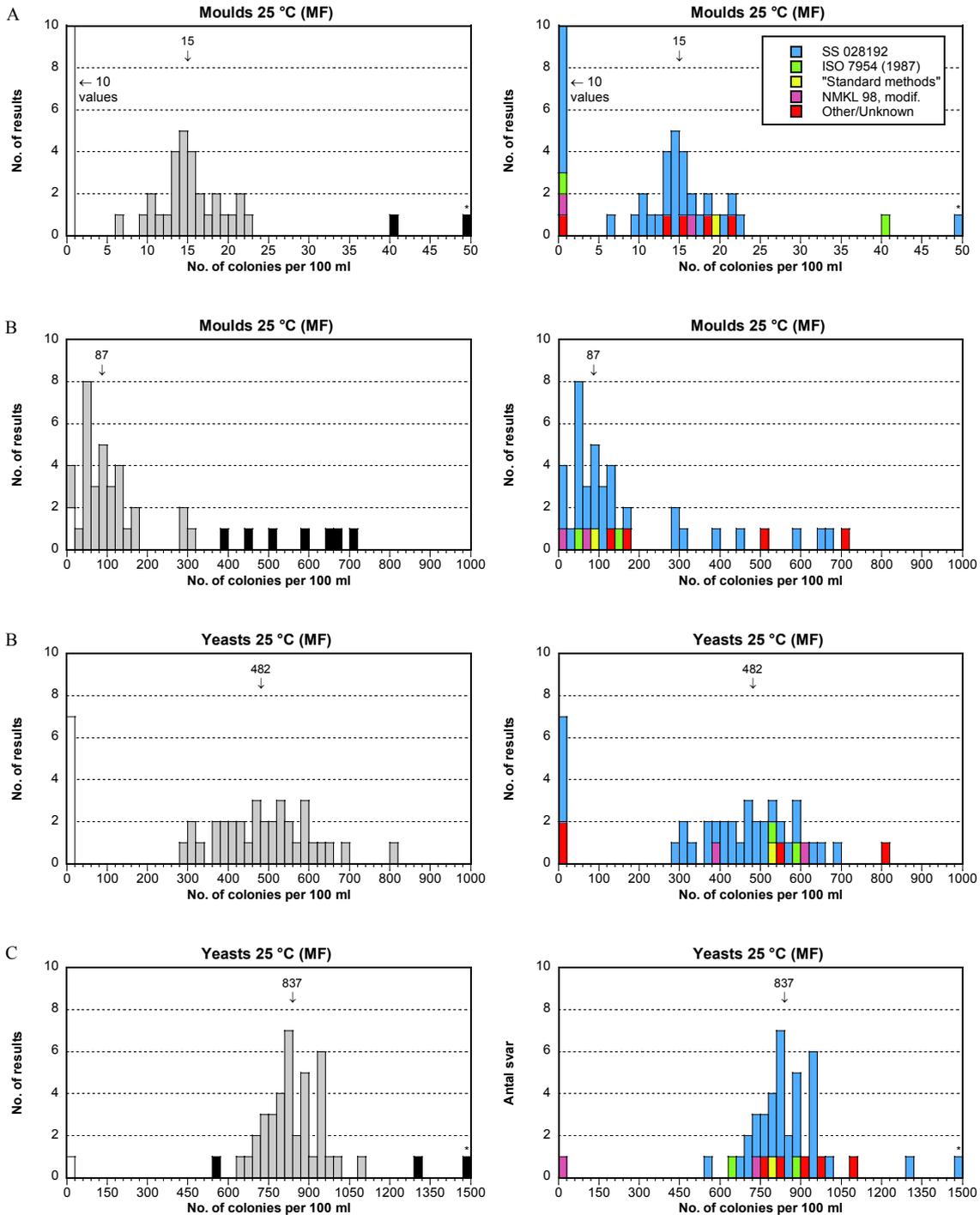
Moulds MF

Standard, Method	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	42	29	15	13	10	0	2	32	87	39	2	0	7	40	0	-	1	-	-
SS 028192	32	23	14	14	7	0	1	26	84	42	1	0	5	30	0	-	1	-	-
ISO 7954 (1987)	2	0	-	-	1	0	1	2	90	-	0	0	0	2	0	-	0	-	-
"Standard methods"	1	1	19	-	0	0	0	1	90	-	0	0	0	1	0	-	0	-	-
NMKL 98, Modif.	2	1	16	-	1	0	0	1	60	-	1	0	0	2	0	-	0	-	-
Other/Unknown	5	4	17	-	1	0	0	2	145	-	0	0	2	5	0	-	0	-	-

Yeasts MF

Standard, Method	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	42	31	0	-	9	-	-	34	482	12	7	0	0	38	837	6	1	1	2
SS 028192	32	24	0	-	6	-	-	27	463	11	5	0	0	29	836	5	0	1	2
ISO 7954 (1987)	2	1	0	-	1	-	-	2	555	-	0	0	0	2	763	-	0	0	0
"Standard methods"	1	1	0	-	0	-	-	1	530	-	0	0	0	1	790	-	0	0	0
NMKL 98, Modif.	2	1	0	-	1	-	-	2	489	-	0	0	0	1	720	-	1	0	0
Other/Unknown	5	4	0	-	1	-	-	2	666	-	2	0	0	5	908	7	0	0	0

The standard SS 028192 was 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 other methods are scattered among those obtained by use of SS 028192. However, several false negative mould and yeast results came from these other methods.

Mixture A

- The mould *Acremonium strictum* was present in the mixture. Microscopy confirms it is a mould. However, it sporulates relatively late and its colonies may therefore look like those from yeasts by its pink appearance on RBCC. This is reflected in the way that 10 laboratories reported false negative results for moulds. Nine of these did at the same time report yeast colonies instead, with the for moulds correct number. These are false positive results.
- Apart from those false negative results, the distribution could be considered as quite good for fungal analyses.

Mixture B

- The mould *Phialophora fastigiata* and the yeast *Issatchenkia orientalis* were present in the mixture. This yeast has a somewhat mould like appearance by having pseudohyphi. It may therefore be taken for a mould. Experience of microscopy of micro fungi is essential for accurate confirmation.
- Seven laboratories reported false negative results for yeasts. These laboratories simultaneously reported high outlying results for moulds. They are in accordance with what the yeast results should have been, which is clear from the histogram.
- The remaining moulds and yeast results have quite good distributions, which show that the laboratories were able to distinguish between moulds and yeasts in an appropriate way.

Mixture C

- The mixture contained the yeast *Candida glabrata*. Except for 4 deviating results, the results distribution was good.

Culturable microorganisms 22 °C, 3 days

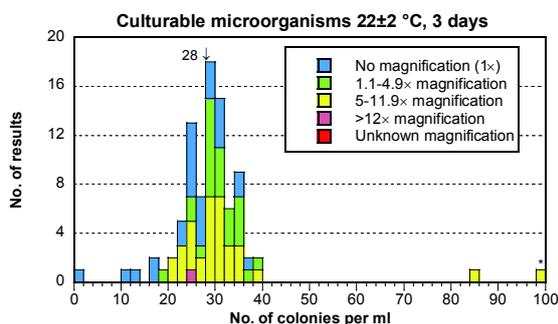
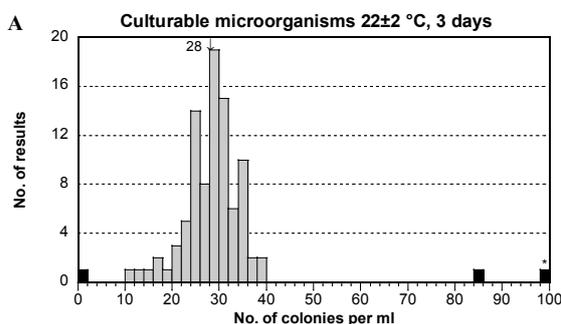
Six of the 94 participating laboratories reported a method other than XX-EN ISO 6222:1999. Four used Plate Count Agar, of which 3 reported "Standard methods" (5). The last two laboratories used Nutrient Agar and Yeast extract agar, respectively, based on other methods. These 6 laboratories reported one high outlier. In mixture B the category "Other method" showed lower average, possibly together with Plate Count Agar.

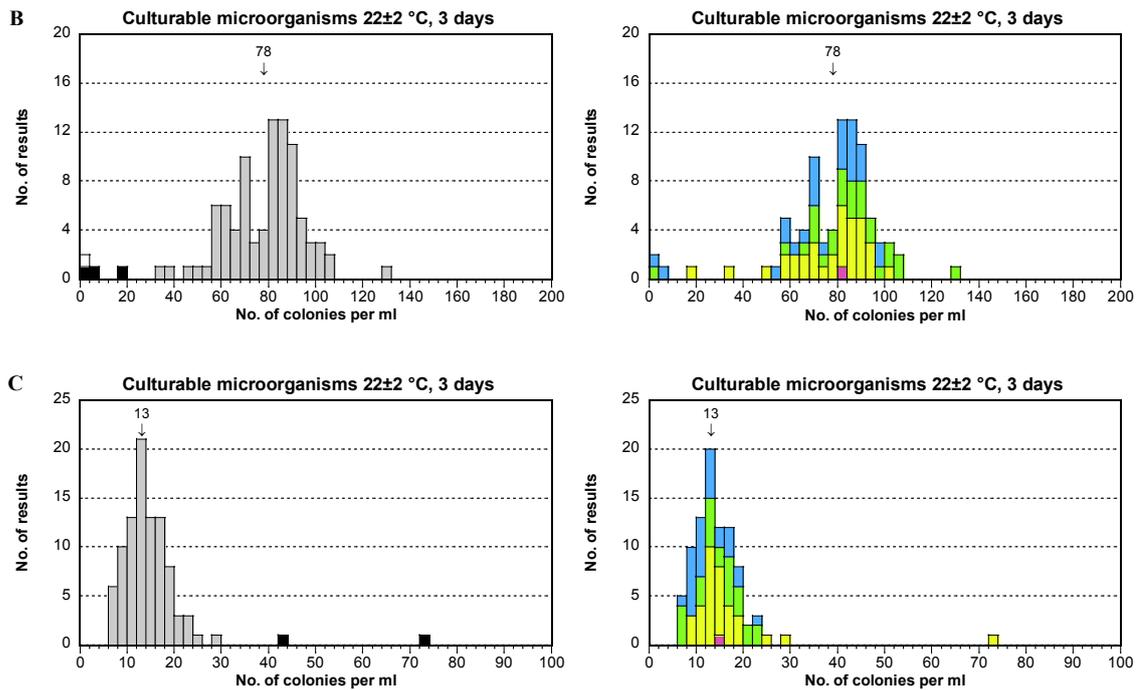
Only comparisons of method variants of the XX-EN ISO 6222:1999 are relevant to discuss. Results are grouped for culture media and magnification for reading.

No general pattern can be discerned in terms of medium, neither for average or dispersion (CV). However, low deviating results have been mainly obtained when low magnification was used.

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	94	90	28	10	0	1	2	89	78	11	1	3	0	92	13	16	0	0	2
<i>EN ISO 6222</i>	88	84	28	10	0	1	2	83	80	9	1	1	0	87	13	16	0	0	0
<i>Medium</i>																			
Yeast extract Agar	83	80	28	10	0	0	2	79	80	10	1	2	0	82	13	16	0	0	1
Plate Count Agar	5	4	26	-	0	1	0	6	72	8	0	1	0	5	16	15	0	0	0
Other/Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<i>Magnification</i>																			
None	27	26	25	13	0	1	0	25	77	8	0	2	0	27	12	15	0	0	0
1,1-4,9×	26	25	30	7	0	0	0	28	85	9	1	0	0	26	14	18	0	0	0
5-11,9×	34	32	28	8	0	0	2	32	77	10	0	1	0	33	14	14	0	0	1
> 12×	1	1	24	-	0	0	0	1	82	-	0	0	0	1	14	-	0	0	0
Unknown	0	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-
<i>Other method</i>	6	6	24	15	0	0	0	6	53	10	0	0	0	5	14	19	0	0	1





Mixture A

- The parameter "culturable microorganisms" is constituted by the two coliform bacteria strains. However, the average colony number was somewhat higher here than for the highest peaks of coliform bacteria by the MF method and rapid method.

Mixture B

- The colonies consist almost entirely of *S. cohnii*.
- A tail with low results was present, of which 4 were deviating. The reason is not obvious.

Mixture C

With exception of the 2 high outliers, the distribution of the results was very good. Yet, the CV is highest in this mixture, 16 compared with 10-11%. This is caused by the low average that with a fixed standard deviation automatically results in a higher CV than with higher average.

- The colonies consist of a mixture of the two coliform bacteria and the yeast *C. glabrata*. However, the average was lower than the sum of these organisms from their individual analyses. This indicates that *C. glabrata* did not appear with its full number after 3 days of incubation for most laboratories. Some laboratories might even have excluded the yeast colonies since they claim to discriminate between yeasts and bacteria. Sixteen laboratories stated that they don't count yeasts and moulds, when they count culturable microorganisms. This is remarkable, since most of the laboratories claim the use of the standard XX-EN ISO 6222:1999, where all appearing microorganisms should be included.

Outcome of the results and laboratory assessment

Assessment of the performance

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

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

General information about reported results

The distributions of results for the respective analysis are shown in histograms. A box plot (see below) gives a summarizing image of all the results of a laboratory, except false results. The number of false results and outliers are given below the plot for each laboratory to summarize its performance. These values are highlighted with bold text and colour background in annex A, where all reported results are compiled. The limit values for lowest and highest accepted results are given for each analyse in the summarizing lines at the end of annex A, together with the measurement uncertainty of the mean.

Mixed up samples or results

When whole samples seem to have been mixed up, the corresponding sample numbers are hatched in annex A. In this round two laboratories seem to have only mixed up two results for culturable microorganisms. There are also 5 laboratories with several deviating results independent on each other. Furthermore, a number of laboratories have several deviating results that are somewhat dependent. All laboratories seem to have calculated the results for the volume asked, i.e. 100 ml for all analyses except for culturable microorganisms where 1 ml is appropriate.

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

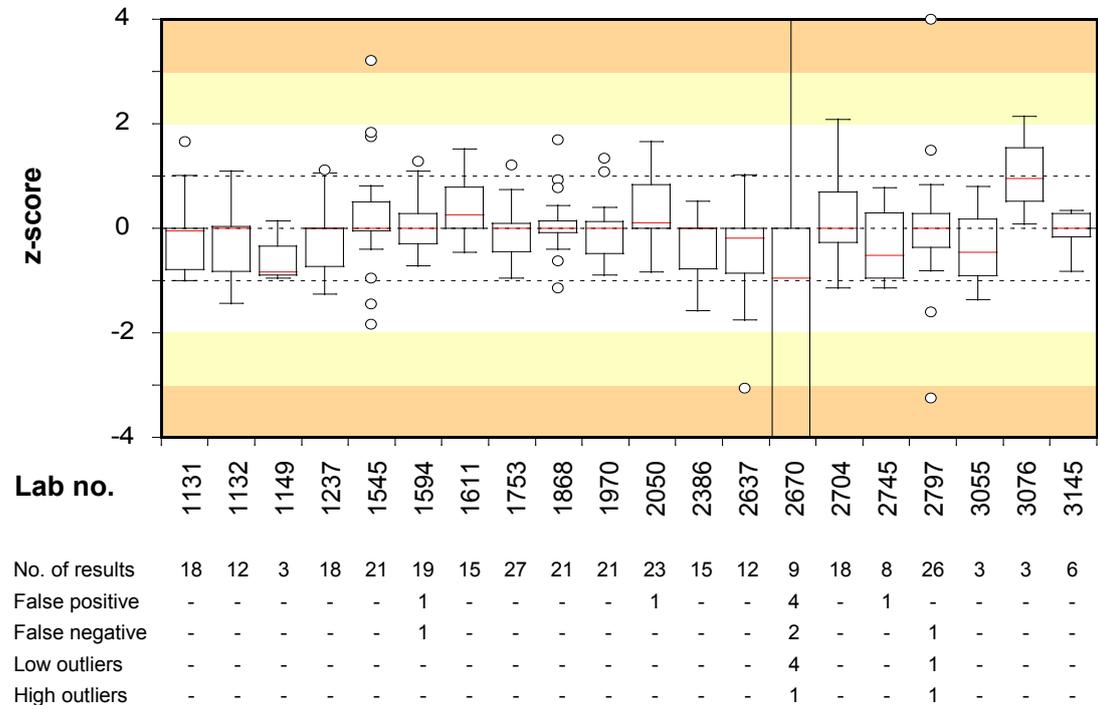
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are shown in annex B but not further evaluated. They are given explicitly to facilitate the follow-up process for the laboratories using z-scores in control charts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

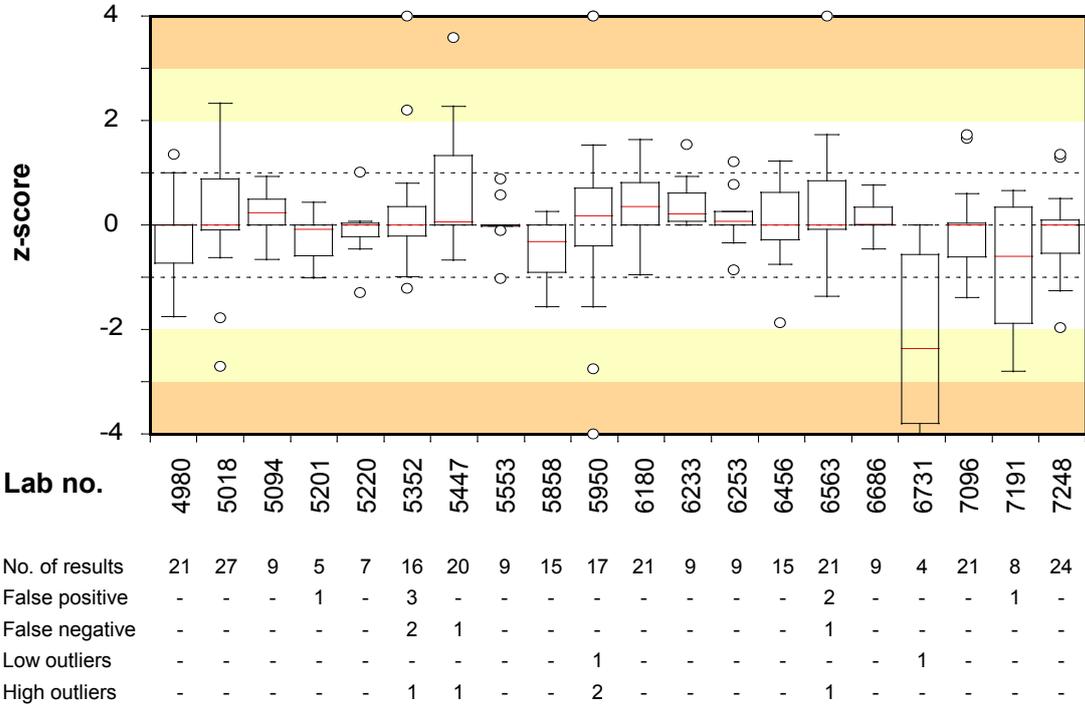
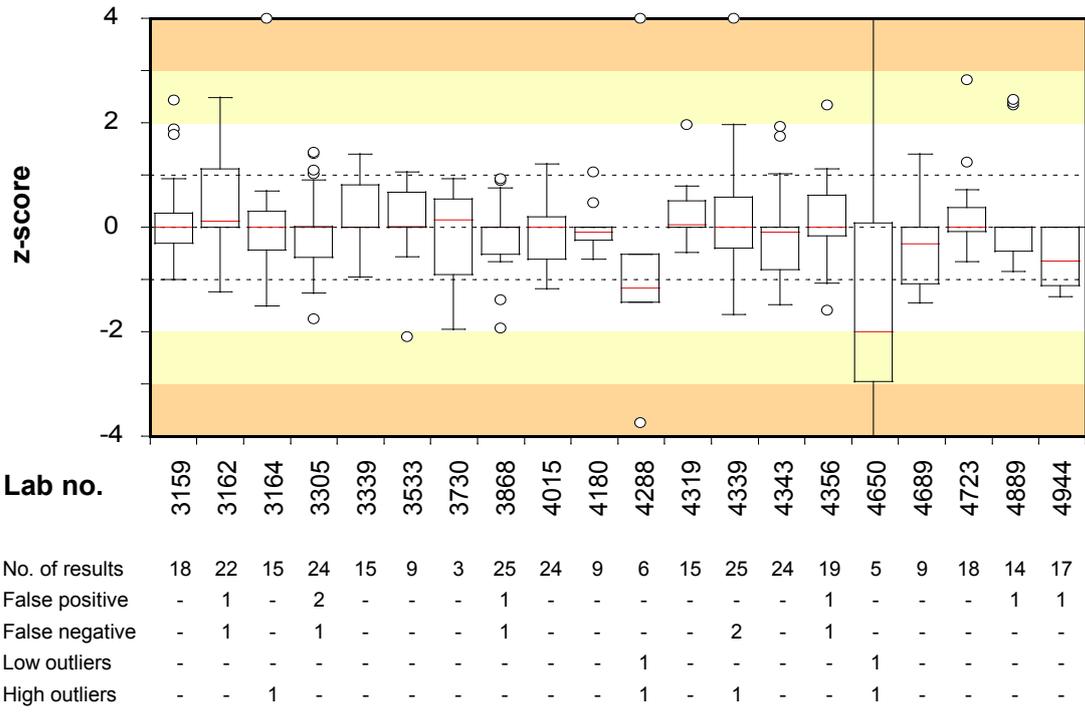
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

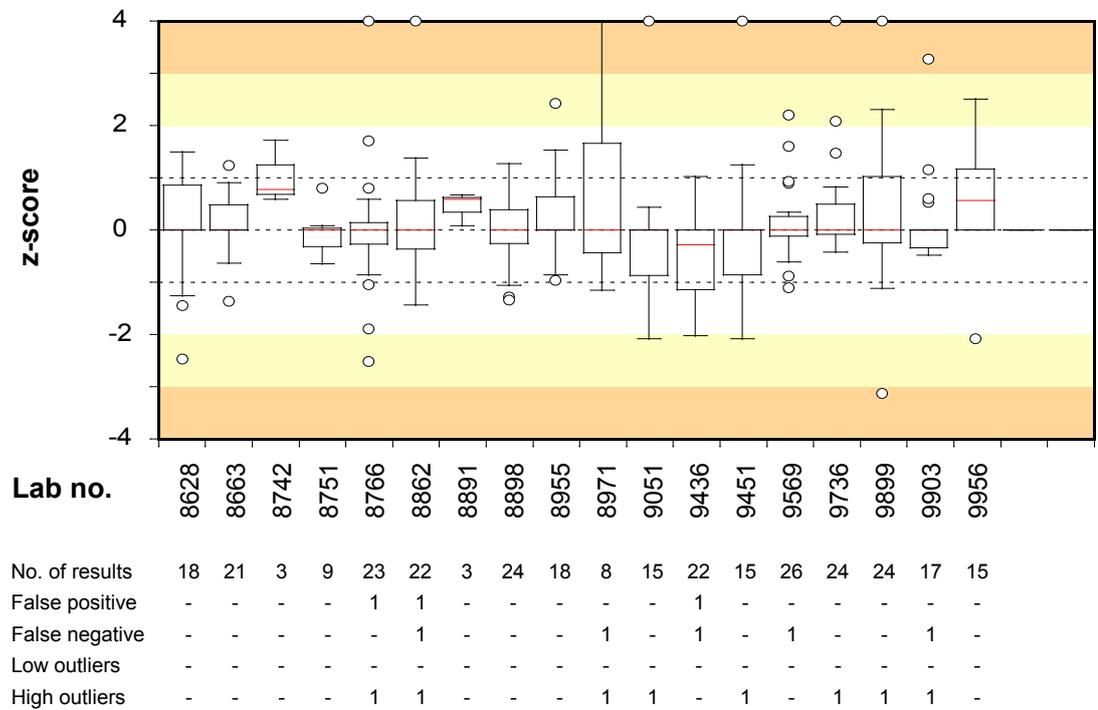
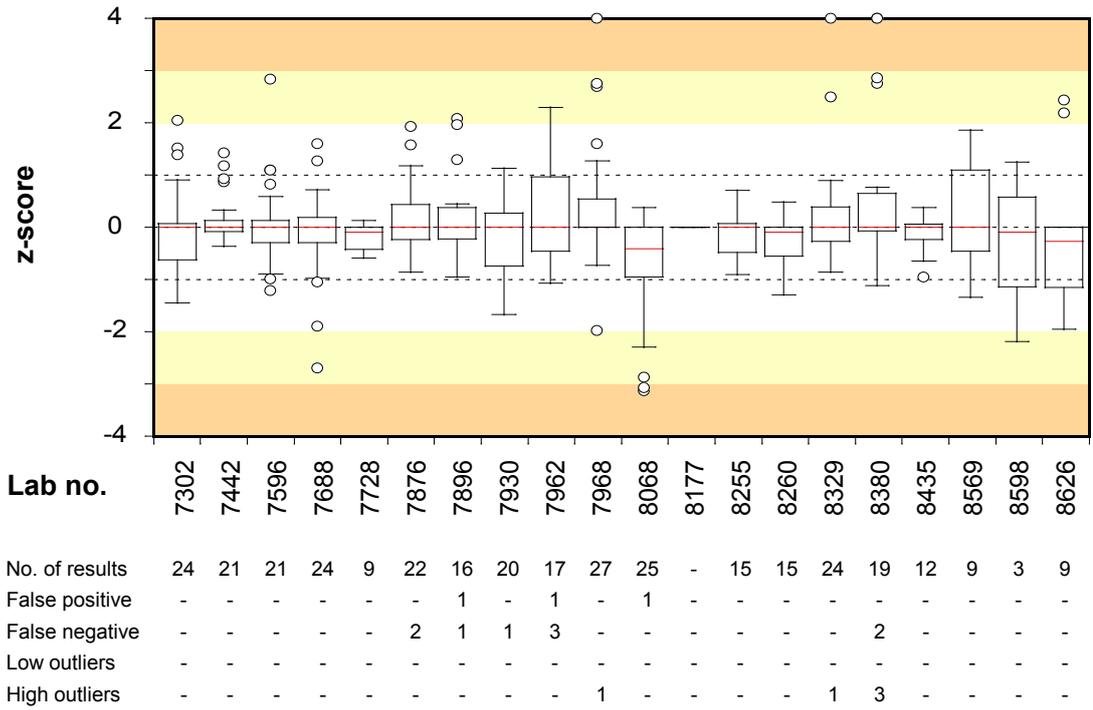
Box plots and numbers of deviating results for each participating laboratory

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

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







Test material, quality controls and processing of data

Description of the test material

The test material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd (2). This round comprised three test items with different microorganism mixtures. 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 by them.

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

Table 2 *Microorganisms present in the mixtures*

Mixture ¹	Microorganisms	Strain no.	cfu/100 ml ²
A	<i>Klebsiella pneumoniae</i>	SLV-186	1000
	<i>Hafnia alvei</i>	SLV-015	2100
	<i>Clostridium perfringens</i>	SLV-442	30
	<i>Acremonium strictum</i>	SLV-502	19
B	<i>Escherichia coli</i>	SLV-165	14
	<i>Aeromonas hydrophila</i>	SLV-533	155
	<i>Phialophora fastigiata</i>	SLV-504	20
	<i>Issatchenkia orientalis</i>	SLV-498	430
	<i>Staphylococcus cohnii</i>	SLV-462	84 *
C	<i>Klebsiella pneumoniae</i>	SLV-186	510
	<i>Klebsiella oxytoca</i>	SLV-089	730
	<i>Clostridium bifermentans</i>	SLV-009	#
	<i>Candida glabrata</i>	SLV-052	820

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

2 cfu = colony forming units

* Indicates cfu per ml

The strain grows very erratic leading to scattered results (false positive for *C. perfringens*)

Quality control of the test material

It is essential to have a homogeneous mixture and a uniform volume in all vials in order to allow comparison of all freeze-dried samples derived from one mixture. The volume was checked by weighing at least 11 vials of each mixture. The largest differences between vials were at most 5 mg for a mixture. The highest accepted difference is 15 mg (3%). Table 3 presents the results from the organizer in the form

of concentration means (cfu) and coefficients of variation (CV) from duplicate analyses of 5 vials from each mixture. All mixtures have been used in a previous round. This was the reason for testing only 5 vials. The results relate to the volume that was used for counting the colonies. According to the criteria for the CVs used they were acceptable for the mixtures to be considered homogenous. The highest accepted CV is normally 25%. For very low colony counts a higher CV is accepted. This was applied for moulds in mixture B.

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 <i>Method standard for analysis</i>	Mixture					
	A		B		C	
	cfu	CV	cfu	CV	cfu	CV
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	31	5 ^b	14	7	124	2 ^a
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	8	19 ^b	6	18	45	7 ^a
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	–	–	–	–	–	–
Presumptive <i>Clostridium perfringens</i> (MF) <i>TSC Agar according to ISO/CD 6461-2:2002</i>	30	4 ^a	–	–	–	– ^d
Moulds (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	19	10	2	46 ^a	–	–
Yeasts (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	–	–	43	4 ^a	41	5 ^c
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	36	6	90	4	22	9

¹ n=5 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, 7, 6 and 5 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 Result for 5 ml

d The results of the false positive strain was high and erratic on the volume used and were not read

– 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 that give better normal distributions by decreasing the significance of the high end “tails”. Very deviating values are still present in most

analyses and are identified as outliers (black bars). False negative results are presented with white bars in the histograms.

Outliers are identified by use of Grubbs' test according to a modification by Kelly (3). A level of 1% is set as the risk to incorrectly assess a result as being an outlier. Although the method is objective, 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 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 m_v . 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.: $u = s/\sqrt{n_{m_v}}$ where n_{m_v} 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.

References

1. Anonymous 2014. Scheme protocol, Microbiology, Drinking water & Food, 3rd ed. National Food Agency, Sweden.
2. Peterz, M., Steneryd, A.-C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *J. Appl. Bacteriol.* 74:143-148.
3. Kelly, K. 1990. Outlier detection in collaborative studies. *J. Assoc. Off. Chem.* 73:58-64.
4. Anonymous 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Official Journal of the European Communities.* 5.12.98, L 330/32-54 (*national translations available*).
5. Standard Methods for the Examination of Water and Wastewater, <http://www.standardmethods.org/>

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

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2 3 1	950	108	970	950	9	970	-	-	-	0	9	0	1120	10	930	0	10	0
1132	2 3 1	-	-	-	-	-	-	-	8	-	0	8	0	953	14	948	0	14	0
1149	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	3 2 1	-	-	-	760	10	980	-	-	-	0	8	0	1300	19	890	0	19	0
1545	2 1 3	3000	109	970	3000	6	970	850	6	460	0	6	0	-	-	-	-	-	-
1594	2 1 3	965	98	910	965	13	910	1015	14	430	0	13	0	1046	14	980	0	14	0
1611	3 1 2	1500	180	960	1500	20	960	1080	11	460	0	20	0	2600	17	980	0	17	0
1753	2 3 1	1014	12	1236	1014	12	1236	-	-	-	0	12	0	996	17	1250	0	17	0
1868	1 3 2	2421	14	1064	925	14	1064	-	-	-	0	14	0	2755	13	1187	0	13	0
1970	1 2 3	1010	114	980	910	9	880	810	14	460	0	14	0	-	-	-	-	-	-
2050	3 1 2	-	-	-	918	16	1318	-	-	-	0	16	0	1923	12	1497	0	12	0
2386	1 3 2	800	16	880	800	16	880	800	16	880	0	16	0	-	-	-	-	-	-
2637	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	2200	11	1000	<1	11	<1
2670	1 2 3	38	42	117	38	42	117	38	42	117	38	42	117	-	-	-	-	-	-
2704	2 1 3	-	-	-	980	21	900	-	-	-	0	21	0	890	25	890	<1	22	<1
2745	2 1 3	960	9	840	960	9	840	960	9	840	0	9	470	-	-	-	-	-	-
2797	3 2 1	900	91	1300	900	16	1300	870	11	390	0	11	0	1000	15	1000	0	15	0
3055	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	993	15	1046	0	15	0
3159	3 2 1	-	-	-	2100	40	810	-	-	-	0	9	0	1652	12.4	1013	<1	11.1	<1
3162	1 2 3	1200	160	1350	1200	28	1350	-	-	-	0	28	0	1850	17	1354	0	17	0
3164	2 3 1	750	110	1000	600	95	900	600	95	450	0	14	0	770	15	1250	0	15	0
3305	2 1 3	1820	131	950	1820	8	950	-	-	-	<1	8	<1	1298	11	1013	<1	11	<1
3339	2 1 3	84	130	880	840	20	880	-	-	-	0	20	0	-	-	-	-	-	-
3533	1 3 2	-	-	-	1100	19	870	-	-	-	0	19	0	-	-	-	-	-	-
3730	1 2 3	0	0	0	-	-	-	800	13	450	-	-	-	-	-	-	-	-	-
3868	2 3 1	850	75	930	850	11	930	850	11	640	0	11	0	1990	8	1298	0	6	0
4015	2 1 3	741	130	850	741	14	850	867	19	336	<1	14	<1	816	15	1200	<1	15	<1
4180	3 2 1	-	-	-	1010	19	930	-	-	-	0	19	0	-	-	-	-	-	-
4288	1 3 2	-	-	-	61	770	880	-	-	-	-	-	-	-	-	-	-	-	-
4319	3 1 2	1115	135	1005	1115	12	1005	980	9	460	0	12	0	2019	17	1160	0	17	0
4339	3 1 2	2150	20	1100	2150	20	1100	900	10	470	0	20	0	1987	7	816	<1	7	<1
4343	2 1 3	2126	80	964	2126	9	964	-	-	-	0	9	0	1733	9	770	0	9	0
4356	3 1 2	500	15	1500	500	15	1500	840	18	470	0	15	0	870	12	1300	0	12	0
4650	3 2 1	-	-	-	>23	3.6	9.2	>23	3.6	9.2	-	-	-	-	-	-	-	-	-
4689	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	690	12	1200	0	12	0
4723	2 3 1	1000	16	982	1000	14	982	-	-	-	0	14	0	-	-	-	-	-	-
4889	2 1 3	24	56	12	1000	11	1500	-	-	-	0	11	1500	980	27	980	0	27	0
4944	2 3 1	770	75	860	770	8	860	-	-	-	0	8	260	780	9	890	0	8	0
4980	3 2 1	1060	80	830	1060	10	830	890	12	520	0	12	0	1298	20.7	831	<1	16.4	<1
5018	1 2 3	1980	90	960	990	36	960	-	-	-	<1	27	<1	1100	13	550	<1	13	<1
5094	2 3 1	2080	84	1080	820	17	1080	710	12	630	0	12	0	-	-	-	-	-	-
5201	1 2 3	730	13	863	-	-	-	-	-	-	730	13	0	-	-	-	-	-	-
5220	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	>200	13.7	>200	<1	13.7	<1
5352	1 3 2	2300	70	960	2300	14	960	1500	14	350	960	0	350	-	-	-	-	-	-
5447	2 1 3	-	-	-	2200	14	1300	-	-	-	0	14	0	-	-	-	-	-	-
5553	3 2 1	-	-	-	685	20	975	-	-	-	<1,0	18	<1,0	-	-	-	-	-	-
5858	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	1400	12	780	0	12	0
5950	3 2 1	3600	135	1191	1181	135	509	972	14	355	-	17	0	2613	14	1211	-	14	-
6180	1 3 2	1952	132	1286	1952	18	1286	1215	12	538	0	18	0	2039	19	1152	0	19	0
6233	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	1609	14	1465	0	14	0
6253	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	973	14	1380	0	14	0
6456	3 1 2	-	-	-	920	21	936	-	-	-	0	21	0	2005	18	697	0	16	0
6563	3 1 2	781	115	1100	781	23	1100	-	-	-	<1	23	660	2382	13	1120	<1	13	<1
6686	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	2005	15	1184	<1	15	<1
6731	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	>1	>1	>1	0	>1	>1
7096	1 2 3	840	96	980	840	10	980	-	-	-	0	10	0	1180	8	1090	0	8	0
7191	3 2 1	620	23	540	620	20.7	540	620	11.5	540	620	16.1	0	-	-	-	-	-	-
7248	2 1 3	809	12	918	809	12	918	580	8	418	0	8	0	980	13	1203	0	12	0
7302	3 1 2	909	127	936	909	11	936	<1	14	536	<1	11	<1	2600	8	1300	<1	8	<1
7442	3 2 1	2200	156	1045	936	14	1045	-	-	-	0	14	0	2518	18	1036	0	18	0
7596	1 3 2	1030	9	1150	1030	9	1150	960	12	360	0	12	0	1120	14	1350	0	14	0
7688	1 3 2	-	-	-	890	16	1000	-	-	-	0	16	0	2400	10	870	0	9	0
7728	3 1 2	-	-	-	875	14	990	-	-	-	0	14	0	-	-	-	-	-	-
7876	1 2 3	1030	110	1400	1030	<10	1400	890	11	410	<1	<10	<1	1046	10	1274	<1	10	<1
7896	3 4 2	810	-	800	810	-	800	940	-	250	<1	-	<1	3100	-	1400	<1	-	<1
7930	2 3 1	1090	109	890	1090	7	890	-	-	-	0	7	0	885	19	831	0	19	0
7962	2 3 1	900	10	900	900	10	900	820	11	280	0	10	0	866	26	866	0	26	0
Mean					1094	15	979				0	13	0	1471	14	1083	0	13	0
CV (%)					20	26	10				-	18	-	22	17	11	-	17	-

table. The mean value (Mean) is the square of the mean value for the square root transformed results (mv). The coefficient of variation (CV) is the standard deviation (s) in percentage of the mean value for the square root transformed results. As means to calculate the z-values of your own, the appropriate values of mv and s are given at the end of the table. The x-values of a laboratory are obtained as the square roots of each reported result, respectively. $z = (x - mv) / s$.

$u_{rel,mv}$ is the relative standard uncertainty of mv in per cent. For calculation see the scheme protocol (1); also briefly described in the text.

Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
490	0	0	-	-	-	-	-	-	-	-	-	29	77	18	1131
-	-	-	-	-	-	-	-	-	-	-	-	34	66	8	1132
-	-	-	80	0	0	-	-	-	-	-	-	23	63	14	1149
-	-	-	510	0	0	13	125	0	0	580	890	30	73	13	1237
510	0	0	-	-	-	0	150	0	15	580	880	34	100	12	1545
-	-	-	-	-	-	-	-	-	-	-	-	29	93	13	1594
-	-	-	-	-	-	-	-	-	-	-	-	30	68	10	1611
153	0	0	153	0	0	14	59	0	0	505	877	30	68	10	1753
-	-	-	-	-	-	15	50	0	0	360	835	33	91	14	1868
-	-	-	-	-	-	19	90	0	0	530	790	24	82	14	1868
140	0	5000	140	0	0	20	40	0	0	518	945	30	95	15	1970
-	-	-	396	0	5100	-	-	-	-	-	-	23	82	12	2050
-	-	-	72	0	0	-	-	-	-	-	-	23	82	12	2386
72	0	0	90	<1	<1	-	-	-	-	-	-	13	81	7	2637
0	18	0	0	18	0	-	-	-	-	-	-	1	7	10	2670
-	-	-	210	0	0	-	-	-	-	-	-	36	85	9	2704
-	-	-	-	-	-	-	-	-	-	-	-	31	91	9	2745
-	-	-	-	-	-	9	580	0	0	0	550	27	91	13	2797
330	0	32	330	0	0	-	-	-	-	-	-	25	57	17	3055
-	-	-	-	-	-	-	-	-	-	-	-	28	94	24	3076
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3145
-	-	-	240	0	0	-	-	-	-	-	-	33	69	22	3159
220	0	4200	-	-	-	0	40	0	14	450	780	21	83	19	3162
-	-	-	-	-	-	-	-	-	-	-	-	25	55	15	3164
360	<1	5	360	<1	5	<1	90	<1	19	480	730	34	103	7	3305
380	0	0	380	0	0	-	-	-	-	-	-	30	85	20	3339
-	-	-	-	-	-	-	-	-	-	-	-	17	89	17	3533
-	-	-	-	-	-	-	-	-	-	-	-	33	49	14	3730
220	0	80	220	0	0	0	50	0	13	460	930	31	80	12	3868
410	<1	14	-	-	-	12	50	<1	<1	425	838	30	86	11	4015
-	-	-	-	-	-	-	-	-	-	-	-	27	68	11	4180
-	-	-	-	-	-	-	-	-	-	-	-	22	58	8	4288
-	-	-	-	-	-	-	-	-	-	-	-	29	70	23	4319
160	0	0	160	0	0	13	660	0	0	0	870	38	0	13	4339
216	0	3727	-	-	-	22	45	0	0	432	775	22	70	12	4343
190	0	3400	0	0	3400	-	-	-	-	-	-	33	97	14	4356
-	-	-	-	-	-	-	-	-	-	-	-	28	37	43	4650
-	-	-	-	-	-	-	-	-	-	-	-	21	61	20	4689
273	0	82	-	-	-	16	136	0	0	473	784	35	90	28	4723
-	-	-	160	0	0	-	-	-	-	-	-	-	-	-	4889
-	-	-	180	0	0	-	-	-	-	-	-	24	70	9	4944
300	0	0	300	0	0	-	-	-	-	-	-	19	95	7	4980
370	<1	3300	370	<1	<1	18	300	<1	<1	300	860	26	80	19	5018
-	-	-	-	-	-	-	-	-	-	-	-	33	82	16	5094
-	-	-	-	-	-	-	-	-	-	-	-	30	62	11	5201
-	-	-	-	-	-	-	-	-	-	-	-	25	58	18	5220
101	0	1400	101	0	1400	14	645	0	0	0	880	26	69	17	5352
600	0	4200	600	0	0	15	500	0	0	0	773	34	98	11	5447
-	-	-	195	<1,0	<1,0	-	-	-	-	-	-	-	-	-	5553
73	0	0	73	0	0	-	-	-	-	-	-	29	66	10	5858
236	-	2700	-	-	-	13	100	-	-	390	691	29	17	72	5950
280	0	0	280	0	0	-	-	-	-	-	-	30	82	17	6180
-	-	-	-	-	-	-	-	-	-	-	-	33	88	16	6233
-	-	-	-	-	-	-	-	-	-	-	-	29	91	12	6253
-	-	-	-	-	-	-	-	-	-	-	-	28	99	12	6456
350	<1	3700	-	-	-	<1	100	<1	19	370	810	420	57	15	6563
-	-	-	-	-	-	-	-	-	-	-	-	25	78	13	6686
-	-	-	-	-	-	-	-	-	-	-	-	11	2	9	6731
490	0	1500	490	0	0	-	-	-	-	-	-	31	85	9	7096
-	-	-	-	-	-	-	-	-	-	-	-	14	59	14	7191
280	0	4800	-	-	-	11	5	0	0	650	855	24	83	13	7248
182	<1	5200	-	-	-	10	282	<1	<1	409	709	20	80	15	7302
-	-	-	-	-	-	15	91	0	0	473	955	26	78	13	7442
101	0	53	101	0	0	-	-	-	-	-	-	34	131	16	7596
-	-	-	210	0	0	6	80	0	0	290	1000	29	90	16	7688
-	-	-	-	-	-	-	-	-	-	-	-	27	71	11	7728
200	<1	4300	-	-	-	17	70	<1	<1	680	830	28	85	11	7876
270	-	<1	270	-	<1	<1	-	<1	14	-	820	26	-	23	7896
-	-	-	230	0	0	-	-	0	-	310	950	-	84	18	7930
-	-	-	-	-	-	<1	<1	<1	15	600	<1	34	86	19	7962
229	0	861	208	0	0	15	87	0	0	482	837	28	78	13	Mean
28	-	105	31	-	-	13	39	-	-	12	6	10	11	16	CV (%)

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7968	1 2 3	1250	139	985	1250	19	985	885	12	410	0	19	0	1048	29	1084	0	29	0
8068	2 3 1	920	26	980	920	14	980	700	2	280	0	14	290	1050	3	620	0	3	0
8177	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8255	3 1 2	1300	95	860	1300	17	860	-	-	-	<10	17	<10	950	12	950	0	12	0
8260	3 1 2	923	14	900	923	11	900	788	11	346	<1	11	<1	-	-	-	-	-	-
8329	2 3 1	986	18	1082	986	18	1082	909	10	336	0	18	0	1300	10	1733	0	10	0
8380	1 2 3	-	-	-	<1	44	770	-	-	-	<1	44	<1	2000	16	860	<1	16	<1
8435	2 1 3	-	-	-	1080	14	800	860	4	300	0	14	0	-	-	-	-	-	-
8569	3 2 1	900	140	730	900	33	730	620	8	360	0	8	0	-	-	-	-	-	-
8598	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8626	1 3 2	950	130	900	660	40	630	285	26	450	0	26	0	-	-	-	-	-	-
8628	1 2 3	-	-	-	1100	6	1300	882	8	509	0	8	0	-	-	-	-	-	-
8663	1 2 3	830	100	920	830	20	920	580	5	320	0	20	0	730	16	1300	0	16	0
8742	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8751	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	1091	12	1091	<1	12	<1
8766	3 2 1	955	174	1145	955	4	1145	1090	9	418	<1	4	<1	1015	10	1053	<1	9	<1
8862	1 2 3	918	144	1273	918	144	1273	-	-	-	0	14	0	2416	12	1093	0	12	0
8891	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8898	2 1 3	890	102	1236	890	14	1236	-	-	-	0	14	0	875	15	1396	0	15	0
8955	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	920	13	1400	0	13	0
8971	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9051	3 2 1	1300	63	610	1300	63	610	820	11	520	0	11	0	-	-	-	-	-	-
9436	1 3 2	854	20	790	854	8	790	636	10	345	<1	8	<1	1744	14	1191	<1	14	<1
9451	1 2 3	860	310	800	860	310	800	0	5	80	0	12	0	-	-	-	-	-	-
9569	1 2 3	2300	15	1000	2300	15	1000	800	<1	290	<1	15	<1	1700	11	1100	<1	11	<1
9736	3 1 2	983	69	1149	983	12	1149	-	-	-	0	12	0	2560	13	1250	0	13	0
9899	1 3 2	2369	145	1081	2369	13	1081	-	-	-	0	13	0	2923	9	1028	0	9	0
9903	2 3 1	1031	110	1088	1031	12	1088	1019	13	429	0	13	0	-	-	-	-	-	-
9956	2 1 3	2500	90	1100	2500	20	1100	1080	15	470	0	20	0	2247	16.1	1203	0	16.1	0

n	62	61	62	75	75	76	43	44	44	75	75	75	61	61	61	62	61	61
Min	0	0	0	0	0	9.2	0	0	9.2	0	0	0	690	3	550	0	3	0
Max	3600	310	1500	3000	770	1500	1500	95	880	960	44	1500	3100	29	1733	0	29	0
Median	962.5	91	967	957.5	14	962	850	11	423.5	0	14	0	1298	13.5	1091	0	13.5	0
Mean				1094	15	979				0	13	0	1471	14	1083	0	13	0
CV (%)				20	26	10				-	18	-	22	17	11	-	17	-
False positive				0	0	0				4	0	7	0	0	0	0	0	0
False negative				1	1	0				0	2	0	0	0	0	0	0	0
Outliers, low				2	0	2				0	0	0	0	0	0	0	0	0
Outliers, high				0	6	0				0	2	0	0	0	0	0	0	0
Low limit OK	0	0	0	500	3	509	0	0	9	0	4	0	690	3	550	0	3	0
High limit OK	3600	310	1500	3000	44	1500	1500	95	880	0	28	0	3100	29	1733	0	29	0

mv				33.081	3.890	31.289				0.000	3.659	0.000	38.355	3.696	32.906	0.000	3.659	0.000
(√Mean)																		
s				6.762	0.997	3.177				0.000	0.658	0.000	8.327	0.627	3.493	0.000	0.628	0.000
(CV*mv/100)																		
U _{rel,mv} (%)				2.4	3.1	1.2					2.1		2.8	2.2	1.4		2.2	
(100*s/√n _{mv})																		
x																		
(√Result)																		
z																		
([x-mv]/s)																		

Presumptive C. <i>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	
210	0	4700	210	0	0	40	45	0	0	530	655	28	88	21	7968
9	0	0	-	0	0	16	60	0	0	390	720	17	64	11	8068
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8177
-	-	-	-	-	-	-	-	-	-	-	-	24	80	13	8255
274	<1	<1	274	<1	<1	-	-	-	-	-	-	24	58	13	8260
249	0	48	-	-	-	54	105	0	0	418	773	30	93	13	8329
-	-	-	-	-	-	14	390	<1	<1	<1	1300	24	83	12	8380
-	-	-	170	0	0	-	-	-	-	-	-	24	84	14	8435
-	-	-	-	-	-	-	-	-	-	-	-	34	75	19	8569
-	-	-	-	-	-	-	-	-	-	-	-	35	46	13	8598
-	-	-	-	-	-	-	-	-	-	-	-	26	60	7	8626
-	-	-	12	0	0	18	160	0	0	545	964	28	75	19	8628
240	0	530	240	0	0	-	-	-	-	-	-	28	87	15	8663
-	-	-	-	-	-	-	-	-	-	-	-	38	91	16	8742
-	-	-	-	-	-	-	-	-	-	-	-	28	77	17	8751
254	<1	6700	-	-	-	14	73	200	<1	482	2500	28	86	16	8766
273	0	4900	-	-	-	0	130	0	15	564	745	24	64	8	8862
-	-	-	-	-	-	-	-	-	-	-	-	28	89	16	8891
327	0	320	-	-	-	10	20	0	0	550	954	30	71	14	8898
-	-	-	250	0	0	21	130	0	0	800	900	31	100	10	8955
-	-	-	-	-	-	13	700	<1	<1	<1	1100	25	60	17	8971
120	0	0	120	0	0	-	-	-	-	-	-	24	84	9	9051
108	<1	3700	-	-	-	<1	4	<1	15	336	663	22	60	9	9436
120	0	0	120	0	0	-	-	-	-	-	-	35	82	6	9451
110	<1	110	110	<1	<1	<1	80	<1	<1	590	930	29	106	11	9569
232	0	28000	-	-	-	15	286	0	0	533	811	28	87	13	9736
200	0	5909	-	-	-	21	160	0	0	622	829	84	35	10	9899
172	0	4233	-	-	-	14	450	0	0	794	0	31	71	12	9903
-	-	-	-	-	-	-	-	-	-	-	-	36	104	6	9956

45	43	45	39	39	40	41	41	41	40	41	42	93	93	94	n
0	0	0	0	0	0	0	0	0	0	0	0	1	0	6	Min
600	18	28000	600	18	5100	54	700	200	19	800	2500	420	131	72	Max
234	0	96	210	0	0	14	85	0	0	481	832.5	28	82	13	Median
229	0	861	208	0	0	15	87	0	0	482	837	28	78	13	Mean
28	-	105	31	-	-	13	39	-	-	12	6	10	11	16	CV (%)
0	1	0	0	1	4	0	0	1	9	0	0	0	0	0	False pos.
1	0	0	2	0	0	10	2	0	0	7	1	0	1	0	False neg.
0	0	0	0	0	0	0	0	0	0	0	1	1	3	0	Outliers <
0	0	1	0	0	0	2	7	0	0	0	2	2	0	2	Outliers >
9	0	0	12	0	0	6	4	0	0	290	655	11	35	6	Low limit
600	0	6700	600	0	0	22	300	0	0	800	1100	38	131	28	High limit

15.140	0.000	29.351	14.422	0.000	0.000	3.809	9.350	0.000	0.000	21.963	28.924	5.244	8.814	3.657	mv
4.229	0.000	30.855	4.444	0.000	0.000	0.506	3.631	0.000	0.000	2.608	1.684	0.536	0.927	0.579	s
4.2	15.8		5.1			2.5	6.9			2.0	0.9	1.1	1.1	1.7	u _{rel,mv} (%)
															x
															z

Annex B Z-scores calculated from the laboratory results. *Susp.* = Suspected on the membrane filters before confirmation. $z = (x - mv) / s$. Z-scores are calculated also for outliers (excluding false negative results) in the same way as ordinary z-scores. From false positive results no z-scores can 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)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2 3 1				-0.334	-0.892	-0.045				0.000	-1.002	0.000	-0.587	-0.851	-0.690	0.000	-0.792	0.000
1132	2 3 1										0.000	-1.262	0.000	-0.899	0.073	-0.606	0.000	0.132	0.000
1149	3 1 2																		
1237	3 2 1				-0.815	-0.730	0.005				0.000	-1.262	0.000	-0.276	1.057	-0.880	0.000	1.115	0.000
1545	2 1 3				3.208	-1.445	-0.045				0.000	-1.838	0.000						
1594	2 1 3				-0.298	-0.285	-0.353				0.000	-0.082	0.000	-0.722	0.073	-0.459	0.000	0.132	0.000
1611	3 1 2				0.835	0.585	-0.096				0.000	1.235	0.000	1.517	0.681	-0.459	0.000	0.740	0.000
1753	2 3 1				-0.183	-0.427	1.217				0.000	-0.297	0.000	-0.816	0.681	0.701	0.000	0.740	0.000
1868	1 3 2				-0.395	-0.148	0.419				0.000	0.125	0.000	1.697	-0.144	0.443	0.000	-0.085	0.000
1970	1 2 3				-0.431	-0.892	-0.511				0.000	0.125	0.000						
2050	3 1 2				-0.412	0.111	1.579				0.000	0.517	0.000	0.660	-0.370	1.656	0.000	-0.311	0.000
2386	1 3 2				-0.709	0.111	-0.511				0.000	0.517	0.000						
2637	3 1 2													1.027	-0.605	-0.368	0.000	-0.546	0.000
2670	1 2 3				-3.981	2.600	-4.000					4.000							
2704	2 1 3				-0.263	0.695	-0.406				0.000	1.402	0.000	-1.024	2.078	-0.880	0.000	1.644	0.000
2745	2 1 3				-0.310	-0.892	-0.726				0.000	-1.002							
2797	3 2 1				-0.456	0.111	1.500				0.000	-0.521	0.000	-0.809	0.282	-0.368	0.000	0.341	0.000
3055	3 1 2																		
3076	3 1 2																		
3145	2 3 1													-0.822	0.282	-0.162	0.000	0.341	0.000
3159	3 2 1				1.885	2.443	-0.890				0.000	-1.002	0.000	0.275	-0.278	-0.309	0.000	-0.522	0.000
3162	1 2 3				0.231	1.407	1.716				0.000	2.479	0.000	0.559	0.681	1.114	0.000	0.740	0.000
3164	2 3 1				-1.270	4.000	-0.406				0.000	0.125	0.000	-1.274	0.282	0.701	0.000	0.341	0.000
3305	2 1 3				1.417	-1.065	-0.147				0.000	-1.262	0.000	-0.280	-0.605	-0.309	0.000	-0.546	0.000
3339	2 1 3				-0.606	0.585	-0.511				0.000	1.235	0.000						
3533	1 3 2				0.013	0.471	-0.564				0.000	1.063	0.000						
3730	1 2 3																		
3868	2 3 1				-0.581	-0.575	-0.250				0.000	-0.521	0.000	0.751	-1.383	0.894	0.000	-1.927	0.000
4015	2 1 3				-0.867	-0.148	-0.672				0.000	0.125	0.000	-1.176	0.282	0.497	0.000	0.341	0.000
4180	3 2 1				-0.192	0.471	-0.250				0.000	1.063	0.000						
4288	1 3 2				-3.737	4.000	-0.511												
4319	3 1 2				0.046	-0.427	0.130				0.000	-0.297	0.000	0.790	0.681	0.330	0.000	0.740	0.000
4339	3 1 2				1.965	0.585	0.591				0.000	1.235	0.000	0.747	-1.674	-1.243	0.000	-1.615	0.000
4343	2 1 3				1.927	-0.892	-0.076				0.000	-1.002	0.000	0.393	-1.109	-1.477	0.000	-1.050	0.000
4356	3 1 2				-1.586	-0.017	2.342				0.000	0.325	0.000	-1.064	-0.370	0.902	0.000	-0.311	0.000
4650	3 2 1					-1.999	-4.000												
4689	3 2 1													-1.452	-0.370	0.497	0.000	-0.311	0.000
4723	2 3 1				-0.216	-0.148	0.015				0.000	0.125	0.000						
4889	2 1 3				-0.216	-0.575	2.342				0.000	-0.521		-0.847	2.391	-0.459	0.000	2.450	0.000
4944	2 3 1				-0.789	-1.065	-0.618				0.000	-1.262		-1.252	-1.109	-0.880	0.000	-1.324	0.000
4980	3 2 1				-0.077	-0.730	-0.780				0.000	-0.297	0.000	-0.280	1.361	-1.168	0.000	0.623	0.000
5018	1 2 3				-0.239	2.117	-0.096				0.000	2.334	0.000	-0.623	-0.144	-2.707	0.000	-0.085	0.000
5094	2 3 1				-0.658	0.234	0.496				0.000	-0.297	0.000						
5201	1 2 3											-0.082	0.000						
5220	2 1 3													0.009			0.000	0.068	0.000
5352	1 3 2				2.200	-0.148	-0.096												
5447	2 1 3				2.044	-0.148	1.500				0.000	0.125	0.000						
5553	3 2 1				-1.022	0.585	-0.020				0.000	0.886	0.000						
5858	1 3 2													-0.113	-0.370	-1.425	0.000	-0.311	0.000
5950	3 2 1				0.190	4.000	-2.747					0.704		1.533	0.073	0.542		0.132	
6180	1 3 2				1.642	0.354	1.439				0.000	0.886	0.000	0.817	1.057	0.296	0.000	1.115	0.000
6233	3 1 2													0.211	0.073	1.537	0.000	0.132	0.000
6253	3 1 2													-0.860	0.073	1.215	0.000	0.132	0.000
6456	3 1 2				-0.407	0.695	-0.219				0.000	-0.755	0.000	0.771	0.871	-1.863	0.000	0.543	0.000
6563	3 1 2				-0.759	0.909	0.591				0.000	1.726		1.255	-0.144	0.160	0.000	-0.085	0.000
6686	3 1 2													0.771	0.282	0.430	0.000	0.341	0.000
6731	2 1 3																0.000		
7096	1 2 3				-0.606	-0.730	0.005				0.000	-0.755	0.000	-0.481	-1.383	0.031	0.000	-1.324	0.000
7191	3 2 1				-1.210	0.662	-2.534					0.536	0.000						
7248	2 1 3				-0.686	-0.427	-0.312				0.000	-1.262	0.000	-0.847	-0.144	0.509	0.000	-0.311	0.000
7302	3 1 2				-0.434	-0.575	-0.219				0.000	-0.521	0.000	1.517	-1.383	0.902	0.000	-1.324	0.000
7442	3 2 1				-0.368	-0.148	0.327				0.000	0.125	0.000	1.420	0.871	-0.206	0.000	0.930	0.000
7596	1 3 2				-0.146	-0.892	0.826				0.000	-0.297	0.000	-0.587	0.073	1.098	0.000	0.132	0.000
7688	1 3 2				-0.480	0.111	0.105				0.000	0.517	0.000	1.277	-0.851	-0.977	0.000	-1.050	0.000
7728	3 1 2				-0.518	-0.148	0.055				0.000	0.125	0.000						
7876	1 2 3				-0.146		1.929				0.000		0.000	-0.722	-0.851	0.798	0.000	-0.792	0.000
7896	3 1 2				-0.683		-0.946				0.000		0.000	2.080		1.291	0.000	0.000	0.000
7930	2 3 1				-0.010	-1.248	-0.458				0.000	-1.540	0.000	-1.034	1.057	-1.168	0.000	1.115	0.000
7962	2 3 1				-0.456	-0.730	-0.406				0.000	-0.755	0.000	-1.072	2.236	-0.996	0.000	2.295	0.000
7968	1 2 3				0.336	0.471	0.030				0.000	1.063	0.000	-0.718	2.692	0.005	0.000	2.751	0.000
8068	2 3 1				-0.407	-0.148	0.005				0.000	0.125		-0.715	-3.130	-2.292	0.000	-3.071	0.000
8177	3 1 2																		
8255	3 1 2				0.440	0.234	-0.618				0.000	0.704	0.000	-0.905	-0.370	-0.597	0.000	-0.311	0.000
8260	3 1 2				-0.399	-0.575	-0.406				0.000	-0.521	0.000						
8329	2 3 1				-0.249	0.354	0.505				0.000	0.886	0.000	-0.276	-0.851	2.498	0.000	-0.792	0.000
8380	1 2 3					2.753	-1.114				0.000	4.000	0.000	0.765	0.485	-1.025	0.000	0.543	0.000
8435	2 1 3				-0.032	-0.148	-0.946				0.000	0.125	0.000						
8569	3 2 1				-0.456	1.861	-1.344				0.000	-1.262	0.000						
8598	2 1 3																		
8626	1 3 2				-1.093	2.443	-1.948				0.000	2.187	0.000						
8628	1 2 3				0.013	-1.445	1.500				0.000	-1.262	0.000						
8663	1 2 3				-0.632														

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.

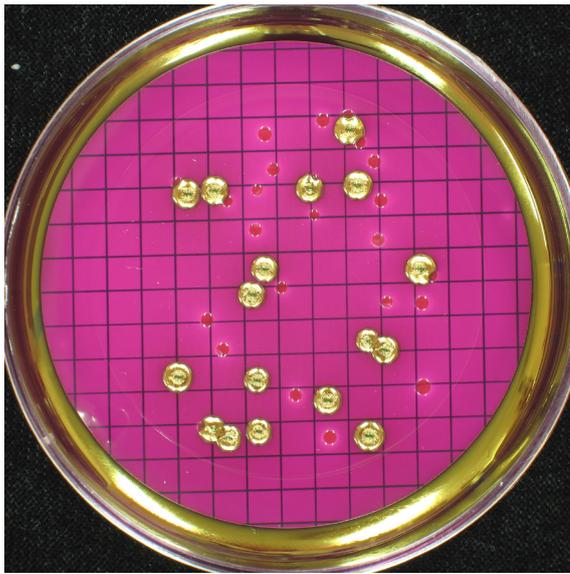
Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
1.654	0.000	-0.951										0.262	-0.042	1.011	1131
								*				1.094	-0.744	-1.430	1132
												-0.837	-0.946	0.146	1149
			-1.233	0.000	0.000							0.434	-0.291	-0.089	1237
1.760	0.000	-0.951	1.837	0.000	0.000	-0.403	0.504	0.000	0.000	0.813	0.540	0.434	0.379	-0.089	1545
							0.798	0.000		0.813	0.440	1.094	1.279	-0.333	1594
												0.262	0.894	-0.089	1611
-0.655	0.000	-0.951	-0.462	0.000	0.000	-0.134	-0.460	0.000	0.000	0.195	0.410	0.434	-0.612	-0.854	1753
						0.126	-0.628	0.000	0.000	-1.146	-0.016	0.933	0.782	0.146	1868
-0.782	0.000	1.340	-0.583	0.000	0.000	1.086	0.038	0.000	0.000	0.406	-0.485	-0.644	0.260	0.146	1970
			1.233	0.000		1.310	-0.833	0.000	0.000	0.306	1.079	0.434	1.006	0.373	2050
-1.574	0.000	-0.951	-1.336	0.000	0.000							-0.837	0.260	-0.333	2386
			-1.111	0.000	0.000							-3.057	0.200	-1.746	2637
		-0.951		0.000	0.000							-4.000	-4.000	-0.854	2670
			0.016	0.000	0.000							1.409	0.437	-1.134	2704
												0.603	0.782	-1.134	2745
0.716	0.000	-0.768	0.843	0.000	0.000	-1.600	4.000	0.000	0.000		-3.250	-0.090	0.782	-0.089	2797
												-0.456	-1.363	0.804	3055
												0.088	0.950	2.144	3076
															3145
			0.241	0.000	0.000							0.933	-0.547	1.784	3159
-0.073	0.000	1.149				-0.833	0.000			-0.288	-0.591	-1.235	0.320	1.211	3162
												-0.456	-1.507	0.373	3164
0.907	0.000	-0.879	1.024	0.000		0.038	0.000			-0.021	-1.131	1.094	1.439	-1.746	3305
1.030	0.000	-0.951	1.141	0.000	0.000							0.434	0.437	1.407	3339
												-2.092	0.668	0.804	3533
												0.933	-1.956	0.146	3730
-0.073	0.000	-0.661	0.092	0.000	0.000	-0.628	0.000			-0.198	0.934	0.603	0.140	-0.333	3868
1.208	0.000	-0.830				-0.682	-0.628	0.000	0.000	-0.517	0.015	0.434	0.495	-0.588	4015
												-0.090	-0.612	-0.588	4180
												-1.033	-1.292	-1.430	4288
												0.262	-0.483	1.966	4319
-0.589	0.000	-0.951	-0.399	0.000	0.000	-0.403	4.000	0.000	0.000		0.340	1.716		-0.089	4339
-0.105	0.000	1.027				1.741	-0.728	0.000	0.000	-0.452	-0.644	-1.033	-0.483	-0.333	4343
-0.321	0.000	0.939	0.000									0.933	1.116	0.146	4356
												0.088	-2.945	4.000	4650
												-1.235	-1.082	1.407	4689
0.327	0.000	-0.658				0.377	0.637	0.000	0.000	-0.082	-0.549	1.253	0.725	2.821	4723
			-0.399	0.000	0.000										4889
			-0.226	0.000	0.000							-0.644	-0.483	-1.134	4944
0.516	0.000	-0.951	0.652	0.000	0.000							-1.652	1.006	-1.746	4980
0.968	0.000	0.911	1.083	0.000	0.000	0.856	2.195	0.000	0.000	-1.780	0.239	-0.271	0.140	1.211	5018
												0.933	0.260	0.592	5094
												0.434	-1.014	-0.588	5201
												-0.456	-1.292	1.011	5220
-1.204	0.000	0.261	-0.984	0.000		-0.134	4.000	0.000	0.000		0.440	-0.271	-0.547	0.804	5352
2.212	0.000	1.149	2.267	0.000	0.000	0.126	3.584	0.000	0.000		-0.666	1.094	1.170	-0.588	5447
			-0.103	0.000	0.000										5553
-1.560	0.000	-0.951	-1.323	0.000	0.000										5858
0.053		0.733				-0.403	0.179			-0.849	-1.566	0.262	-0.744	-0.854	5950
0.377	0.000	-0.951	0.520	0.000	0.000							0.434	0.260	0.804	6180
												0.933	0.611	0.592	6233
												0.262	0.782	-0.333	6253
												0.088	1.225	-0.333	6456
0.844	0.000	1.020				0.179	0.000			-1.046	-0.275	4.000	-1.363	0.373	6563
												-0.456	0.019	-0.089	6686
												-3.596	-4.000	-1.134	6731
1.654	0.000	0.304	1.736	0.000	0.000							0.603	0.437	-1.134	7096
												-2.803	-1.222	0.146	7191
0.377	0.000	1.294				-0.974	-1.959	0.000	0.000	1.354	0.188	-0.644	0.320	-0.089	7248
-0.390	0.000	1.386				-1.279	2.050	0.000	0.000	-0.667	-1.364	-1.441	0.140	0.373	7302
						0.126	0.052	0.000	0.000	-0.082	1.176	-0.271	0.019	-0.089	7442
-1.204	0.000	-0.715	-0.984	0.000	0.000							1.094	2.837	0.592	7596
			0.016	0.000	0.000	-2.688	-0.112	0.000	0.000	-1.892	1.603	0.262	0.725	0.592	7688
												-0.090	-0.418	-0.588	7728
-0.236	0.000	1.174				0.620	-0.271	0.000	0.000	1.577	-0.068	0.088	0.437	-0.588	7876
0.305		-0.951	0.452		0.000			0.000	0.000			-0.271		1.966	7896
			0.167	0.000	0.000			0.000		-1.670	1.127		0.379	1.011	7930
								0.000		0.971		1.094	0.495	1.211	7962
-0.154	0.000	1.271	0.016	0.000	0.000	4.000	-0.728	0.000	0.000	0.406	-1.978	0.088	0.611	1.597	7968
-2.871	0.000	-0.951		0.000	0.000	0.377	-0.442	0.000	0.000	-0.849	-1.242	-2.092	-0.878	-0.588	8068
															8177
												-0.644	0.140	-0.089	8255
0.334	0.000	-0.951	0.480	0.000	0.000							-0.644	-1.292	-0.089	8260
0.151	0.000	-0.727				4.000	0.247	0.000	0.000	-0.582	-0.666	0.434	0.894	-0.089	8329
						-0.134	2.864	0.000	0.000		4.000	-0.644	0.320	-0.333	8380
			-0.312	0.000	0.000							-0.644	0.379	0.146	8435
												1.094	-0.166	1.211	8569
												1.253	-2.191	-0.089	8598
												-0.271	-1.152	-1.746	8626
			-2.466	0.000	0.000	0.856	0.909	0.000	0.000	0.530	1.262	0.088	-0.166	1.211	8628
0.083	0.000	-0.205	0.241	0.000	0.000							0.088	0.553	0.373	8663
												1.716	0.782	0.592	8742

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
8751	3	2	1																			
8766	3	2	1				-0.322	-1.896	0.802				0.000	-2.520	0.000	-0.640	-0.370	0.036	0.000	-0.311	0.000	
8862	1	2	3				-0.412	4.000	1.382				0.000	0.125	0.000	-0.780	-0.851	-0.131	0.000	-1.050	0.000	
8891	2	1	3													1.297	-0.370	0.044	0.000	-0.311	0.000	
8898	2	1	3				-0.480	-0.148	1.217				0.000	0.125	0.000	-1.054	0.282	1.276	0.000	0.341	0.000	
8955	2	1	3													-0.964	-0.144	1.291	0.000	-0.085	0.000	
8971	3	1	2																			
9051	3	2	1				0.440	4.000	-2.074				0.000	-0.521	0.000							
9436	1	3	2				-0.571	-1.065	-1.002				0.000	-1.262	0.000	0.409	0.073	0.459	0.000	0.132	0.000	
9451	1	2	3				-0.555	4.000	-0.946				0.000	-0.297	0.000							
9569	1	2	3				2.200	-0.017	0.105				0.000	0.325	0.000	0.345	-0.605	0.074	0.000	-0.546	0.000	
9736	3	1	2				-0.256	-0.427	0.821				0.000	-0.297	0.000	1.470	-0.144	0.701	0.000	-0.085	0.000	
9899	1	3	2				2.306	-0.285	0.500				0.000	-0.082	0.000	1.887	-1.109	-0.242	0.000	-1.050	0.000	
9903	2	3	1				-0.144	-0.427	0.534				0.000	-0.082	0.000							
9956	2	1	3				2.502	0.585	0.591				0.000	1.235	0.000	1.087	0.504	0.509	0.000	0.563	0.000	
n				0	0	0	74	74	76	0	0	0	71	73	68	61	61	61	62	61	61	
Min							-3.981	-1.999	-4.000				0.000	-2.520	0.000	-1.452	-3.130	-2.707	0.000	-3.071	0.000	
Max							3.208	4.000	2.342				0.000	4.000	0.000	2.080	2.692	2.498	0.000	2.751	0.000	
Median							-0.328	-0.148	-0.096				0.000	0.125	0.000	-0.280	0.009	0.036	0.000	0.068	0.000	
Mean							-0.104	0.324	-0.105				0.000	0.110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
SD							1.170	1.458	1.178				0.000	1.185	0.000	1.000	1.000	1.000	0.000	1.000	0.000	
z<-3							2	0	2				0	0	0	0	1	0	0	0	1	0
-3<=z<-2							0	0	3				0	1	0	0	0	2	0	0	0	0
-2<=z<=3							5	5	2				0	3	0	1	4	1	0	3	0	0
z>3							1	6	0				0	2	0	0	0	0	0	0	0	0

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	
0.189	0.000	1.702				-0.134	-0.222		0.000	-0.003	4.000	0.088	-0.042	0.804	8751
0.327	0.000	1.317					0.565	0.000		0.685	-0.967	0.088	0.495	0.592	8766
												-0.644	-0.878	-1.430	8862
0.696	0.000	-0.372				-1.279	-1.343	0.000	0.000	0.571	1.166	0.088	0.668	0.592	8891
			0.313	0.000	0.000	1.528	0.565	0.000	0.000	2.424	0.639	0.434	-0.418	0.146	8898
						-0.403	4.000	0.000	0.000		2.520	0.603	1.279	-0.854	8955
-0.990	0.000	-0.951										-0.456	-1.152	0.804	8971
-1.123	0.000	1.020	-0.780	0.000	0.000		-2.024	0.000		-1.393	-1.886	-0.644	0.379	-1.134	9051
-0.990	0.000	-0.951	-0.780	0.000	0.000							-1.033	-1.152	-1.134	9436
-1.100	0.000	-0.611	-0.885	0.000	0.000							1.253	0.260	-2.085	9451
0.022	0.000	4.000					-0.112	0.000	0.000	0.892	0.934	0.262	1.597	-0.588	9569
-0.236	0.000	1.540				0.126	2.083	0.000	0.000	0.431	-0.265	0.088	0.553	-0.089	9736
-0.479	0.000	1.157				1.528	0.909	0.000	0.000	1.142	-0.078	4.000	-3.125	-0.854	9899
						-0.134	3.267	0.000	0.000		-0.443	0.603	-0.418	-0.333	9903
												1.409	1.492	-2.085	9956
44	42	45	37	38	36	31	39	40	31	34	41	93	92	94	n
-2.871	0.000	-0.951	-2.466	0.000	0.000	-2.688	-2.024	0.000	0.000	-1.892	-3.250	-4.000	-4.000	-2.085	Min
2.212	0.000	4.000	2.267	0.000	0.000	4.000	4.000	0.000	0.000	2.424	4.000	4.000	2.837	4.000	Max
0.037	0.000	-0.611	0.016	0.000	0.000	0.126	0.179	0.000	0.000	-0.012	-0.016	0.088	0.230	-0.089	Median
0.000	0.000	0.089	0.000	0.000	0.000	0.258	0.659	0.000	0.000	0.000	0.116	0.043	-0.130	0.085	Mean
1.000	0.000	1.154	1.000	0.000	0.000	1.390	1.700	0.000	0.000	1.000	1.405	1.220	1.215	1.147	SD
0	0	0	0	0	0	0	0	0	0	0	1	3	4	0	Sum
1	0	0	1	0	0	1	1	0	0	0	0	3	2	2	14
1	0	0	1	0	0	0	4	0	0	1	1	0	1	2	17
0	0	1	0	0	0	2	6	0	0	0	2	2	0	2	35
															24

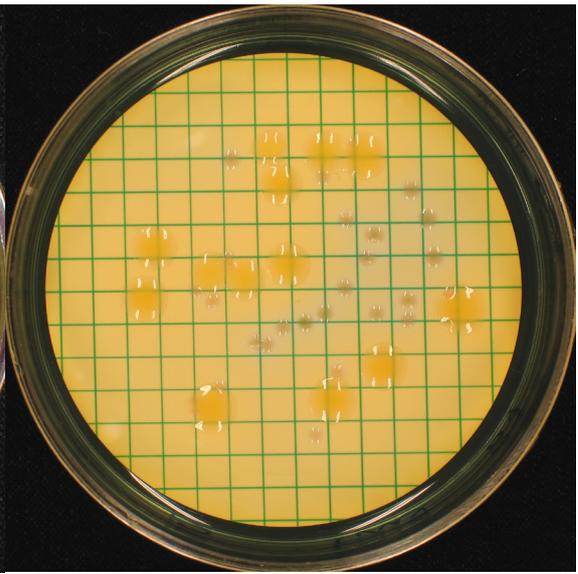
Mixture A

m-Endo Agar LES, 37 °C



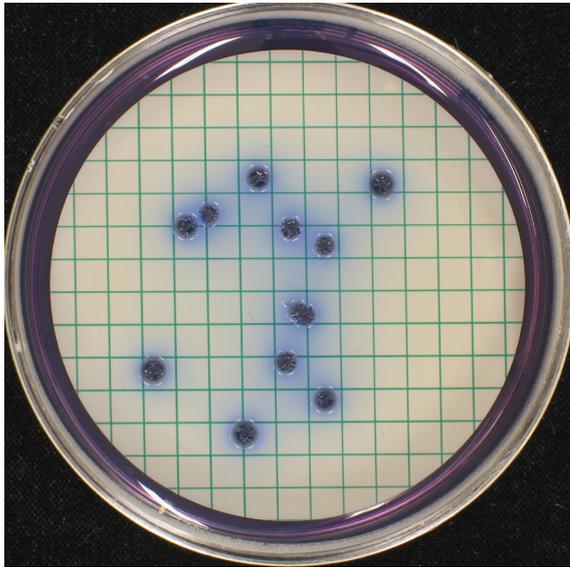
1 ml

m-Lactose TTC Agar, 37 °C



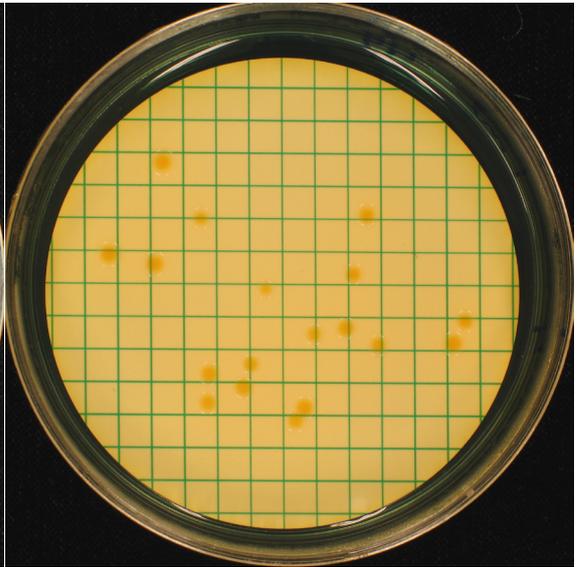
1 ml

m-FC Agar, 44 °C



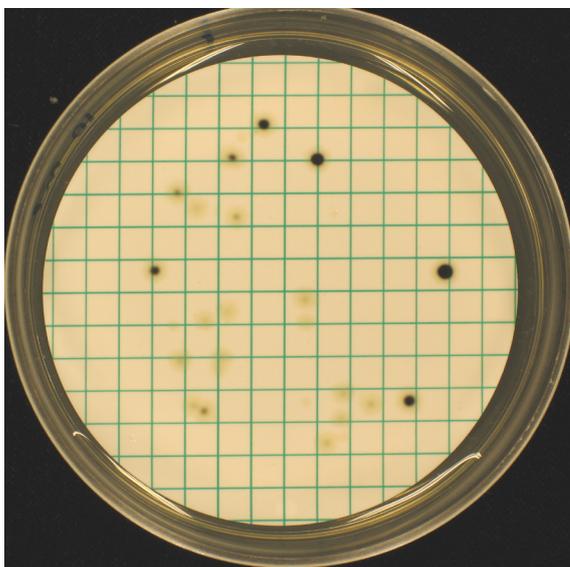
1 ml

m-Lactose TTC Agar, 44 °C



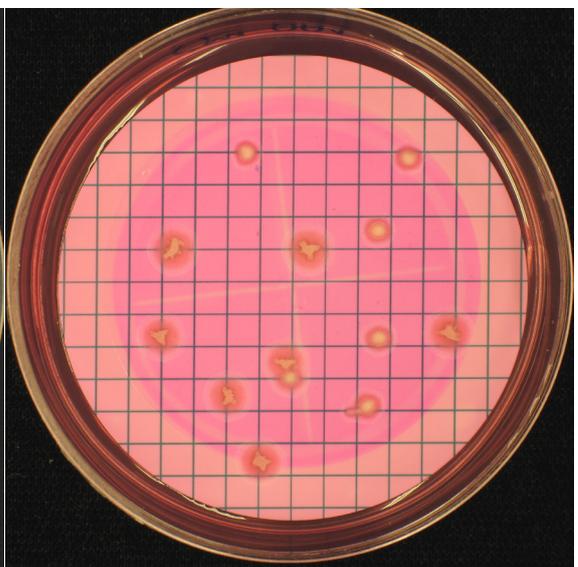
1 ml

m-TSC Agar, 44 °C



10 ml

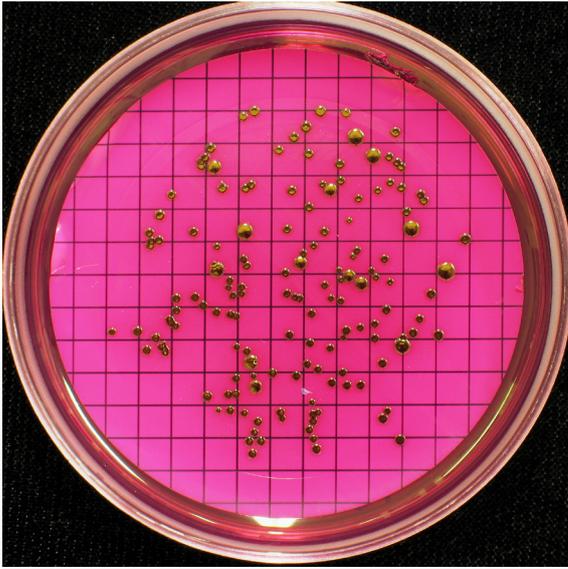
m-RBCC Agar, 25 °C



100 ml, 7 days

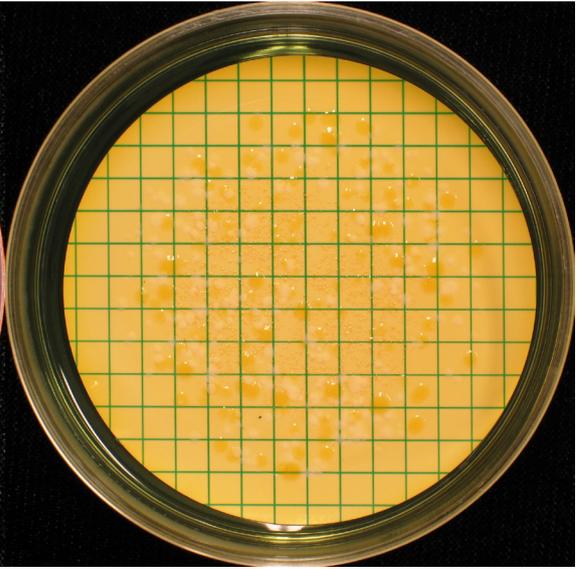
Mixture B

m-Endo Agar LES, 37 °C



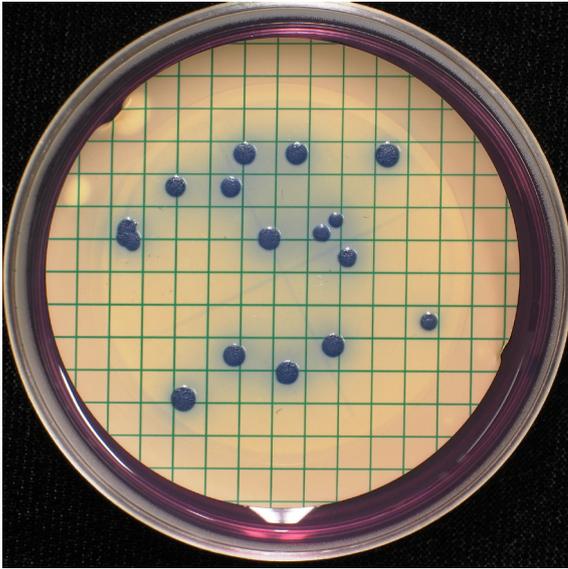
100 ml

m-Lactose TTC Agar, 37 °C



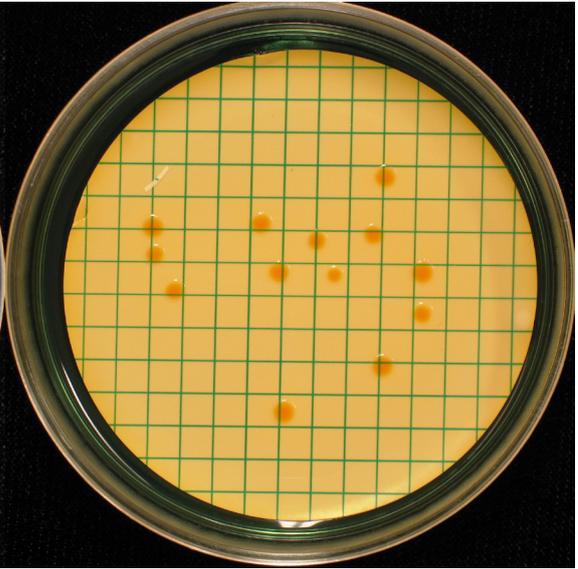
100 ml

m-FC Agar, 44 °C



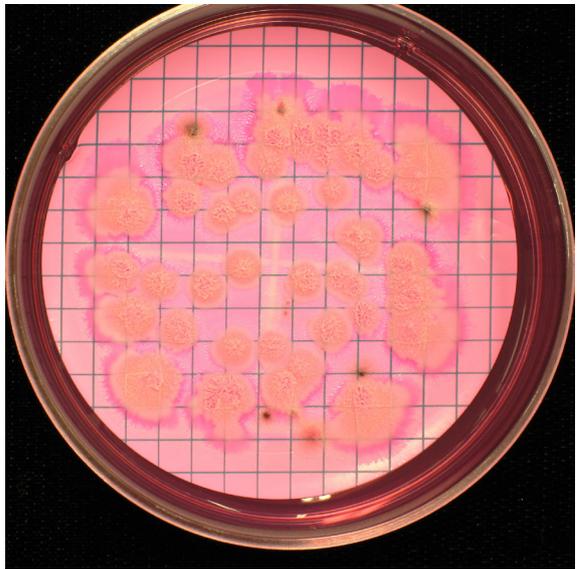
100 ml

m-Lactose TTC Agar, 44 °C



100 ml

m-TSC Agar, 44 °C

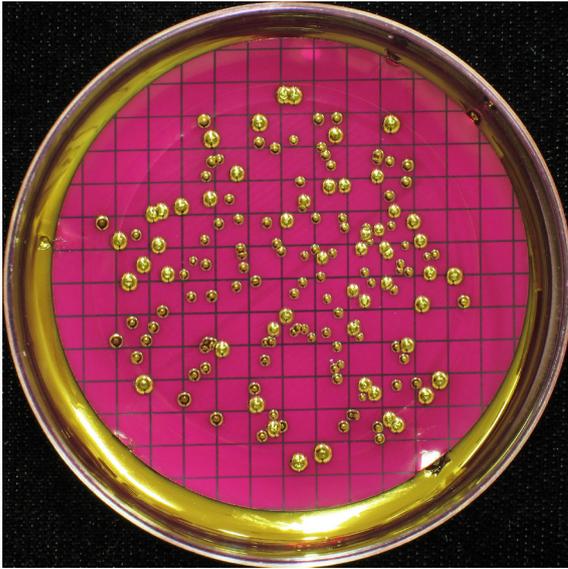


10 ml, 7 days

m-RBCC Agar, 25 °C

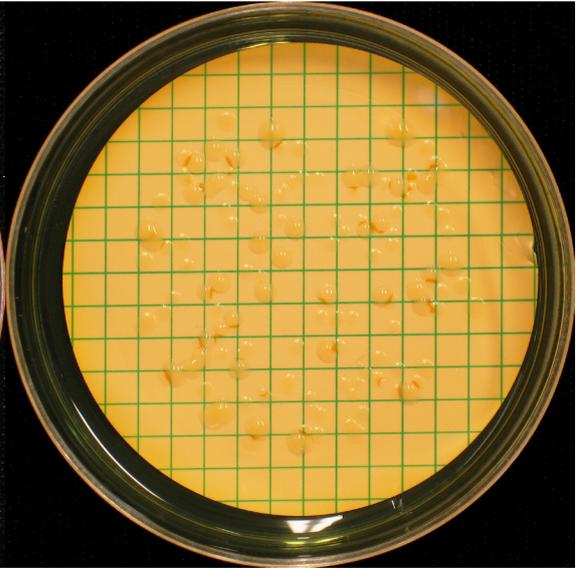
Mixture C

m-Endo Agar LES, 37 °C



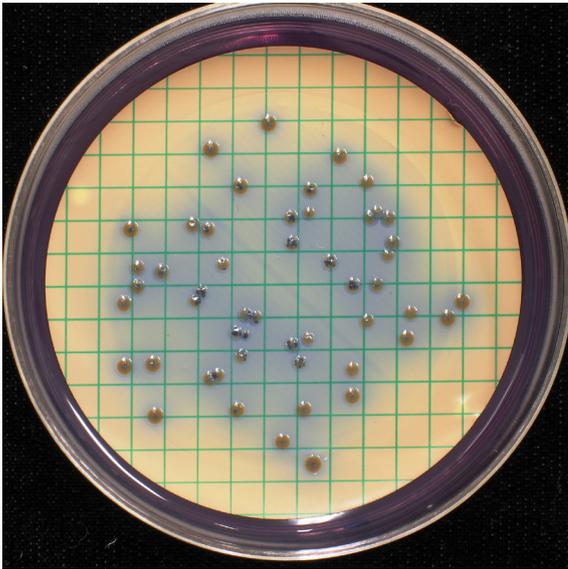
10 ml

m-Lactose TTC Agar, 37 °C



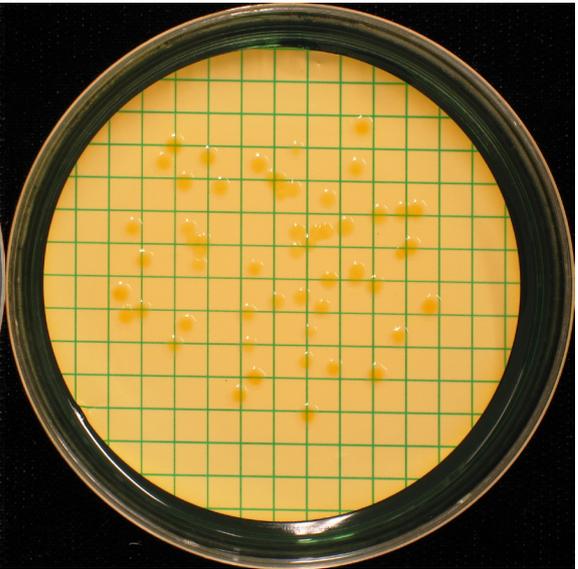
10 ml

m-FC Agar, 44 °C



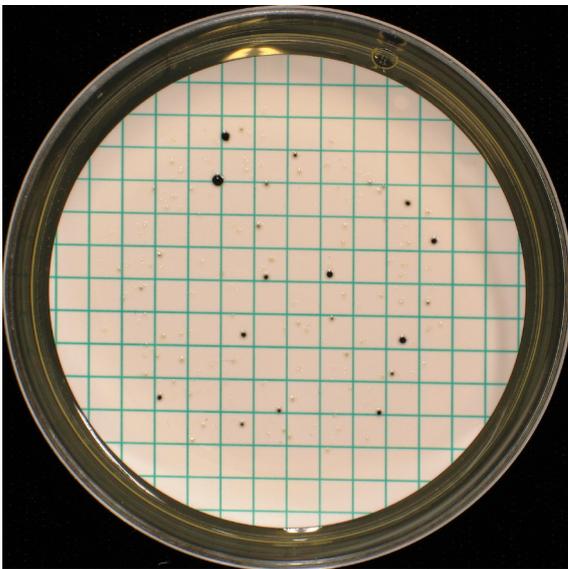
10 ml

m-Lactose TTC Agar, 44 °C



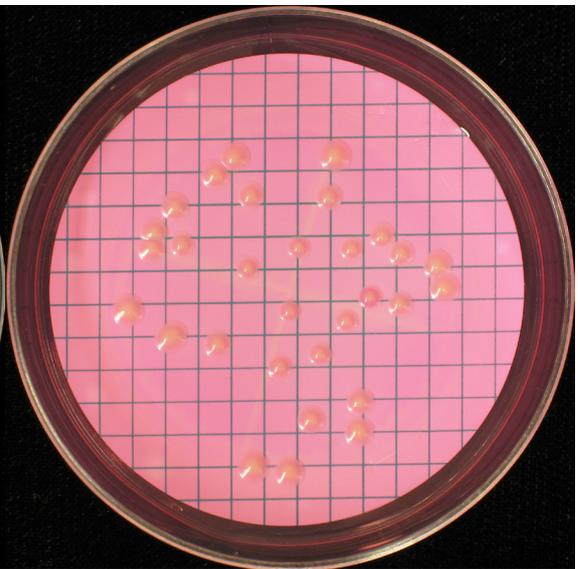
10 ml

m-TSC Agar, 44 °C



100 ml

m-RBCC Agar, 25 °C



5 ml, 7 days

PT reports published 2014

Proficiency Testing – Food Microbiology, January 2014, by Laurence Nachin,
Christina Normark and Irina Boriak

Proficiency Testing – Drinking Water Microbiology, March 2014, by Tommy
Šlapokas and Kirsi Mykkänen

Proficiency Testing – Food Microbiology, April 2014, by Laurence Nachin and Irina
Boriak

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

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

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

The National Food Agency's PT program offers

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

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



1457
ISO/IEC 17043

The National Food Agency's reference material

As a complement to the proficiency testing, National Food Agency also produces reference material (RM) for internal quality control: a total of 8 RM for food and drinking water microbiological analyses, including pathogens, are available.

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