Proficiency Testing

Drinking Water Microbiology

March 2018

Tommy Šlapokas

Livsmedelsverket
National Food Agency

NFA PT
Since 1981

Edition
Version 1 (2018-05-29)

Editor in chief
Hans Lindmark, Head of Biology department, National Food Agency

Responsible for the scheme
Tommy Šlapokas, Microbiologist, Biology department, National Food Agency

PT March 2015 is registered as no. 2017/00791 at the National Food Agency, Uppsala

Proficiency testing

Drinking water Microbiology

March 2018

SWEDAC · ACKREDITERING ·
1457
ISO/IEC 17043

Parameters included

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

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

Clostridium perfringens with MF

Actinomycetes with MF

Moulds with MF

Yeasts with MF

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

Tommy Šlapokas

Linnea Blom & Ramiyeh Molaei

National Food Agency, Biology department, Box 622, SE-751 26 Uppsala, Sweden

Abbreviations and explanations

Microbiological media

ACTA	Actinomycete Isolation Agar (according to SS 028212)
CCA	Chromocult Coliform Agar® (Merck; according to EN ISO 9308-1:2014)
Colilert	Colilert® Quanti-Tray® (IDEXX Inc.; according to EN ISO 9308-2:2014)
LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (acc. to EN-ISO 9308-1:2000)
m-FC	m-FC Agar (according to SS 028167)
PAB/TSC/SFP	Tryptose Sulfite Cycloserine Agar (acc. to EN ISO 14189:2016)
RBCC	Rose Bengal Agar with both chlortetracycline and chloramphenicol (according to SS 028192)
YeA	Yeast extract Agar (according to EN ISO 6222:1999)

Other abbreviations

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

Legend to method comparison tables

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

Explanations to histograms with accepted and deviating results

▨	result without remark
□	false negative result
■	outlier
↓ 34	average without deviating results
*	over a bar means that the result is beyond the x-axis limit

Contents

General information on results evaluation

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

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

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

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

Results of the PT round

General outcome

Test items were sent to 88 laboratories, 36 in Sweden, 49 in other Nordic countries (Faeroe Islands, Greenland and Åland included), 2 more from EU, 1 from the rest of Europe but no one from countries outside Europe. Results were reported from 85 laboratories.

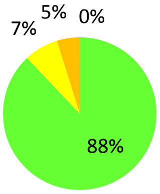
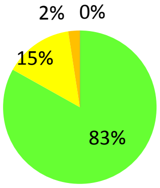
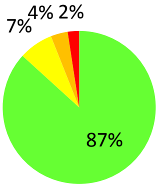
The percentages of false results and outliers are compiled in **table 1**.

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

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

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

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

Mixture	**A**			**B**			**C**		
Percentage of laboratories with 🟩 0 deviating results 🟨 1 deviating result 🟧 2 deviating results 🟥 >2 deviating results									
No. of evaluable results	529			525			507		
No. of deviating results *	14 (3 %)			15 (3 %)			18 (4 %)		
Microorganisms	*Escherichia coli* *Aeromonas hydrophila* *Candida glabrata* *Phialophora fastigiata* *Stenotrophomonas maltophilia*			*Citrobacter freundii* *Klebsiella oxytoca* *Clostridium perfringens* *Streptomyces* sp. *Staphylococcus saprophyticus*			*Escherichia coli* *Enterobacter cloacae* *Hafnia alvei* *Clostridium bifermentans* *Phoma glomerata*		
Analysis	**Target org.**	**F%**	**X%**	**Target org.**	**F%**	**X%**	**Target org.**	**F%**	**X%**
Coliform bacteria (MF)	*E. coli* *[A. hydrophila]*	2	5	*C freundii* *K. oxytoca*	0	5	*E. coli* *E. cloacae*	0	3
Susp. thermotolerant coliform bact. (MF)	*E. coli*	–	–	–	–	–	*E. coli* *{E. cloacae}*	–	–
E. coli (MF)	*E. coli*	2	5	–	7	–	*E. coli* *[E. cloacae]*	30	2
Coliform bacteria (rapid method)	*E. coli*	0	0	*C freundii* *K. oxytoca*	0	0	*E. coli* *E. cloacae*	0	5
E. coli (rapid meth.)	*E. coli*	0	0	–	0	0	*E. coli*	2	0
Presumptive *C. perfringens* (MF)	–	2	–	*C. perfringens*	0	2	*C. bifermentans*	10	0
Clostridium perfringens (MF)	–	3	–	*C. perfringens*	3	0	*[C. biferment.]*	9	–
Actinomycetes (MF) 25 °C	–	0	–	*Streptomyces* sp.	0	0	–	3	–
Moulds (MF) 25 °C	*Ph. fastigiata*	2	0	–	7	–	*Ph. glomerata*	2	0
Yeasts (MF) 25 °C	*C. glabrata*	0	5	–	2	–	–	5	–
Culturable micro-organisms (total count), 3 days 22 °C	*S. maltophilia* *E. coli* *A. hydrophila*	1	0	*S. saprophyticus* *(C. freundii)* *(K. oxytoca)*	0	1	*H. alvei* *E. coli* *E. cloacae*	0	0

* In total 27 of 85 laboratories (32%) reported at least one deviating result

– Organism missing or numerical result irrelevant

( ) The organism contributes with only very few colonies

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

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

Coliform bacteria (MF)

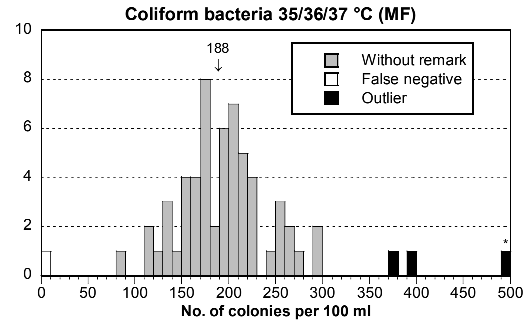
The group "LES, wrong standard" that has been present in earlier PT rounds has now been deleted after communication with those laboratories. The results are now allocated to other groups, mainly m-Endo Agar LES (LES). The only laboratory in the group Other/Unknown has used Tryptone Glucose Extract agar (TGE) and incubated 7 days in room temperature.

From the table it is clear that LES is still used by most laboratories. The proportion that used CCA has continued to increase since the standard EN ISO 9308-1 from 2014 was launched. The use of LTTC from the previous edition of that standard simultaneously decreased and is not reported at all here.

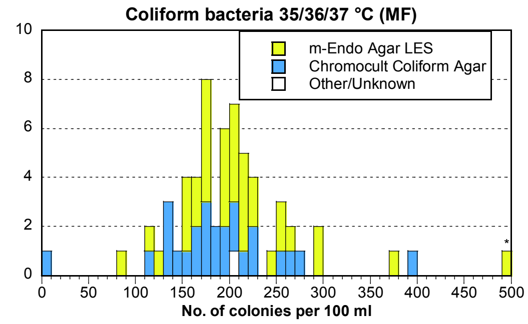
It appears as if LES gave a higher mean result compared to CCA for all samples. In total six different coliform bacteria were present in the samples.

Medium	N	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	61	57	**188**	12	1	0	3	57	**45**	13	0	2	1	57	**3552**	14	0	2	0
m-Endo Agar LES	35	33	**192**	12	0	0	2	33	**49**	12	0	1	1	33	**3852**	10	0	1	0
Chromocult C Agar	25	23	**183**	11	1	0	1	23	**40**	13	0	1	0	23	**3129**	18	0	1	0
Lactose TTC Agar	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
Other/Unknown	1	1	–	–	0	0	0	1	–	–	0	0	0	1	–	–	0	0	0

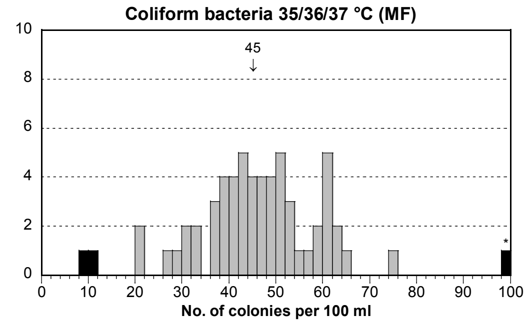
A



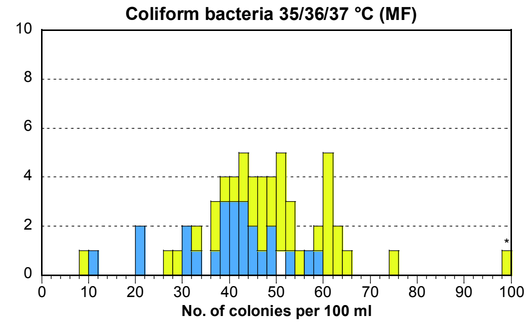
A

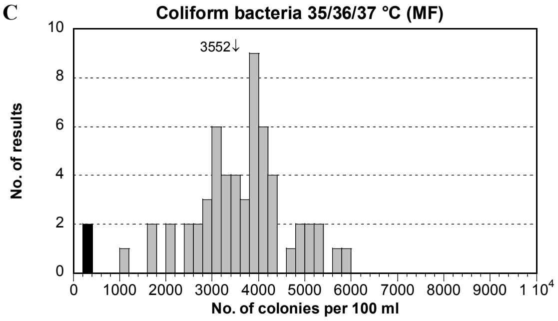


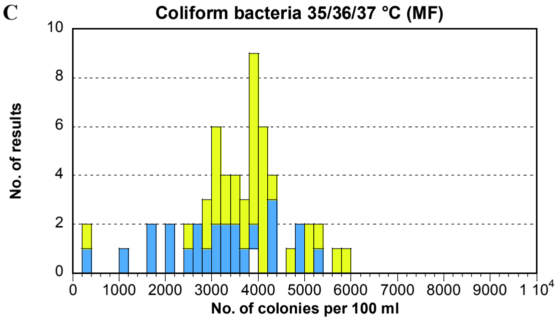
B



B







Mixture A

- A strain of *E. coli* and a strain of *A. hydrophila* were included. They appeared with for coliform bacteria typical colonies on the MF media at 37 °C, a metallic sheen on LES and blue and dark pinkish red, respectively, on CCA.
- The distribution of the results was good with a small dispersion (CV; see page 30). One false negative result and 3 high outliers were present.
- *A. hydrophila* was a false negative strain but could be removed after confirmation with oxidase test because it is oxidase positive. At least one of the high outliers is probably caused by *A. hydrophila* not being removed after confirmation.

Mixture B

- No *E. coli* but strains of two other coliform bacteria, *C. freundii* and *K. oxytoca*, were present. These strains appeared with typical colonies at 37 °C, i.e. with metallic sheen on LES and pink on CCA. There were also some other small pink colonies present on CCA, making it a bit more difficult to see and count the coliform bacteria.
- Despite the background flora, the distribution of the accepted results was fairly good and the dispersion was small. Two low and 2 high outliers were present.
- The average result for CCA was considerably lower than for LES according to the table and can be seen in the histogram as the blue colour in the lower half.

Mixture C

- One strain each of *E. coli* and *E. cloacae* was included together with a strain of *H. alvei* as coliform bacteria. At the National Food Agency (NFA) the two first mentioned grew with distinct colonies on the MF media at 37 °C, a typical metallic sheen on LES and "pink" on CCA. Thus, the colonies of *E. coli* were not blue on CCA but pink with a more or less clear hue of violet in the middle. The colonies of *H. alvei* were red without the metallic sheen on LES and apricot pinkish on CCA. Thus, the colonies should be counted as coliforms on CCA but not on LES. However, the results rather indicate the opposite, as they are lower from CCA in average. Which colonies are included from the two media probably differs among the laboratories.
- Two low outliers were present. The distribution was good and the dispersion low in average.

Suspected thermotolerant coliform bacteria (MF)

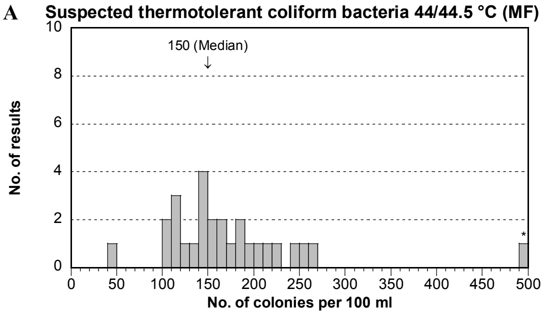
No evaluation in relation to performance is done for what is called suspected (not confirmed) colonies of a parameter. Therefore, no identification of outliers is done. The *medians* are then more robust than the means and are given in the table and in histograms.

Previously, the two most used growth media have been m-FC and LTTC. The incubation temperature is 44 or 44.5 °C. This time no result for LTTC was reported. Because all old method details were previously cleared from the database, and since it is now not mandatory to report method details for suspected organisms, very few details have been reported. This makes an evaluation meaningless and thus no grouping by method is given. ***The parameter is not included in the performance assessment***.

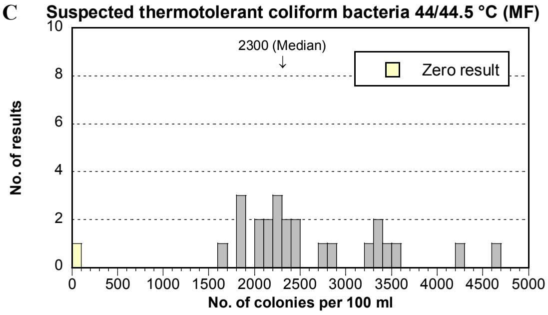
Standard, Method	Tot	A						B						C					
	n	n	**Med**	CV	F	<	>	n	**Med**	CV	F	<	>	n	**Med**	CV	F	<	>
Total	27	27	**150**	–	–	–	–	27	**0**	–	–	–	–	25	**2300**	–	–	–	–

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

A



C



Mixture A

- One strain of *E. coli* together with a strain of *A. hydrophila* appears on media for coliform bacteria at 35-37 °C. Only *E. coli* grows with, for (suspected) thermotolerant coliform bacteria, typical blue colonies on m-FC agar at 44/44.5 °C

- The distribution of the results was good in general. One high result that could be seen as an outlier was present.

Mixture B

- There were no thermotolerant coliform bacteria in the mixture. However, one false positive result was present.

Mixture C

- Three coliform bacteria were included in the mixture, of which the *E. coli* strain appears as a typical suspected thermotolerant coliform bacterium at 44 °C, meaning blue colonies on m-FC. Also the strain of *E. cloacae* may grow with small blue colonies on m-FC, which then should be added to the result.
- The analysis was without problem. One zero result was obtained.
- The distribution of the 25 results possibly seems to have two peaks. This may be an effect of that some laboratories have seen and included the colonies of *E. cloacae*, while others have not done that.

Escherichia coli (MF)

To identify and quantify *E. coli,* confirmation is required when colonies are isolated from the primary cultivation media LES, LTTC and m-FC. Depending on the method, test of indole production and/or β-glucuronidase activity from oxidase negative presumptive strains is usually used. A violet to blue colony on CCA indicates positive β-glucuronidase activity and is reckoned as a confirmed *E. coli*.

The primary growth media CCA, LES as well as LTTC are used at 36±2 °C and LTTC or m-FC at 44/44.5 °C. This time there were no results reported for LTTC based on the standard ISO 9308-1:2000 but instead for CCA from ISO 9308-1:2014. The results are separated in groups based on the standard that was used. For the standards from the Nordic countries (SS, SFS, NS) the majority of the results are

All results

Origin &Standard	**N**	**A**						**B**						**C**					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	62	58	**187**	12	1	1	2	57	**0**	–	4	–	–	41	**2330**	14	18	1	0
Colony origin																			
36 ± 2 °C	45	43	**188**	12	1	0	1	41	**0**	–	3	–	–	30	**2278**	15	13	1	0
44/44.5 °C	8	7	**172**	7	0	0	1	8	**0**	–	0	–	–	6	**2503**	12	2	0	0
36 ± 2 & 44/44.5 °C	9	8	**192**	17	0	1	0	8	**0**	–	1	–	–	5	**2445**	11	3	0	0
Other/Unknown	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
Standard																			
ISO 9308-1:2000	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
ISO 9308-1:2014	26	25	**180**	13	1	0	0	24	**0**	–	1	–	–	14	**2051**	17	10	1	0
SS 028167	15	14	**199**	7	0	0	1	15	**0**	–	0	–	–	12	**2359**	12	3	0	0
SFS 4088	16	16	**190**	16	0	0	0	13	**0**	–	3	–	–	11	**2720**	12	4	0	0
NS 4792	2	2	–	–	0	0	0	2	**0**	–	0	–	–	2	–	–	0	0	0
Other/Unknown	3	1	–	–	0	1	1	3	**0**	–	0	–	–	2	–	–	1	0	0

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

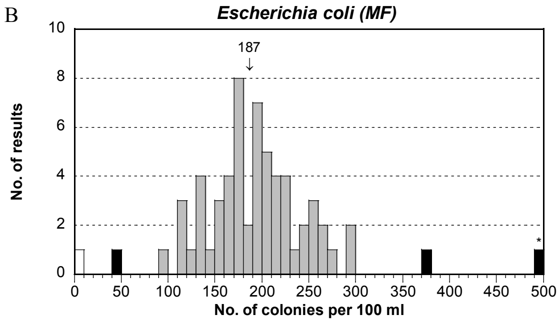
Medium	N	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	*47*[#]	*45*	*189*	*12*	*1*	*0*	*1*	*43*	*0*	*–*	*3*	*–*	*–*	*31*	*2269*	*15*	*14*	1	*0*
m-Endo Agar LES	21	20	**200**	11	0	0	1	19	**0**	–	2	–	–	18	**2463**	13	3	0	0
Lactose TTC Agar	0	–	**–**	–	–	–	–	0	**–**	–	–	–	–	0	**–**	–	–	–	–
Chromocult C Agar	25	24	**179**	13	1	0	0	23	**0**	–	1	–	–	13	**2013**	17	10	1	0
Other/Unknown	1	1	**–**	–	–	–	–	1	**0**	–	0	–	–	0	**–**	–	1	0	0

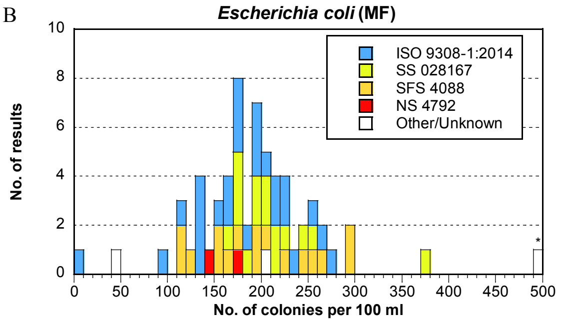
\# Compare table above – two more laboratories performed the analysis of *E. coli* than of coliform bacteria

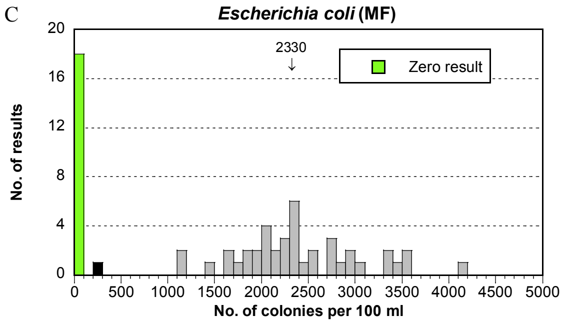
from 36±2 °C on LES but some are also from 44/44.5 °C on m-FC. The results are additionally grouped based on the reported incubation temperature.

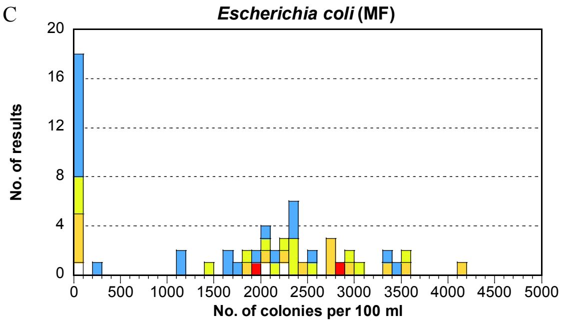
When all results are compared for mixture A, in principle no differences can be seen between the different standards or incubation temperatures, possibly somewhat lower at 44/44.5 °C. However, for mixture C there seems to be differences between the standards. The methods both with CCA and LES have delivered several zero results, the most with CCA; see discussion below about mixture C. Even the average for accepted results is somewhat lower for CCA and the dispersion (CV) is larger.

These differences for all results of mixture C are seen also when only the results from 36±2 °C are compared. Similar differences between LES and CCA have been seen also in previous rounds and need to be further considered with different bacterial strains.









Mixture A

- One typical strain of *E. coli* was present together with another coliform-like bacterium, *A. hydrophila*. The latter is oxidase positive, indole negative, and has no activity of *β*-glucuronidase and does not grow at 44° C. Thus, it cannot be taken for *E. coli* after confirmation.
- The distribution of the results was good and the dispersion small. One false negative result as well as 1 low outlier and 2 high outliers were present.

Mixture B

- No *E. coli* was included but two other coliform bacteria, out of which one was a strain of *K. oxytoca*. That strain is able to grow in broth at 44 °C and is indole positive. That makes a false positive result possible when colonies are picked from plates incubated at 36±2 °C and the indole test alone is used as criterion for *E. coli*.
- Four false positive results were reported.

Mixture C

- A strain of *E. coli* with weak *β*-glucuronidase activity was included together with two other coliform bacteria, *E. cloacae* and *H. alvei*. Sometimes small blue colonies of *E. cloacae* can appear on m-FC at 44 °C. The colony appearance for *E. coli* is typical on LES and m-FC that are based on lactose fermentation. However, on the chromogenic enzyme based medium CCA the colony colour is atypical for *E. coli*. The colonies are there pinkish with a more or less evident violet hue in the middle. It seems that these colonies are often interpreted as coming from another coliform bacterium than *E. coli*, leading to a zero result for *E. coli*. Confirmation for *E. coli* is not considered necessary on CCA and is therefore normally not used. However, confirmation is necessary to discern *E. coli* from other coliforms for colonies picked from LES and m-FC.
- Eighteen zero results were reported together with one low outlier. The distribution was otherwise in general fairly good with a small dispersion (CV = 18 %). The outlier could be caused by a missed calculation of the result for the volume 100 ml. In the histogram the average is given for the results except the zero results and the only outlier.
- Ten of the 18 zero results were obtained by using CCA, all but one of the other by using the Nordic standards based on lactose fermentation and confirmation.
- The strain of *E. coli* is producing gas in lactose broth at 44 °C, is positive when testing for indole production but show a weak *β*-glucuronidase activity. That the outcome is interpreted as negative is probably seen when *β*-glucuronidase activity is the only decisive criterion for *E.coli*. This is applicable to confirmation with MUG reagent in broth as well as to the use of enzyme based chromogenic media like CCA.
- **Zero results obtained due to an interpretation of the *β*-glucuronidase activity as negative is acceptable** even though they are indicated as false negative in the table and Annex A. However, zero results by other reasons should be seen as real false negative ones.

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

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

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

A difference based on the maximum incubation length is often small. However, for coliform bacteria in mixture C there might be one; the maximum 22 hours gave somewhat higher average results than the maximum 20 hours. Tendencies to similar behaviour are seen also for coliform bacteria and *E. coli* in mixture A. There are only few results for the group "24 hours" but the average is specified as it is low. In one case the method specified is Colilert 24. Due to the few results it is impossible to make any conclusions.

There is nothing in the evaluation that suggests that there was any problem with interpretation of the results.

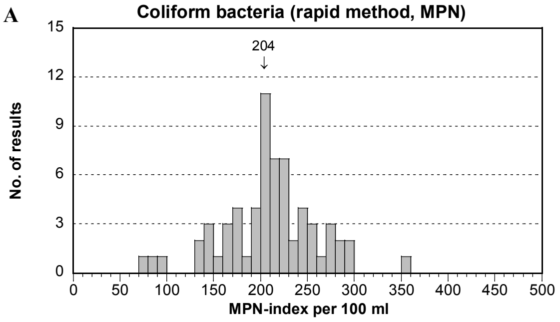
Coliform bacteria, Rapid method with MPN

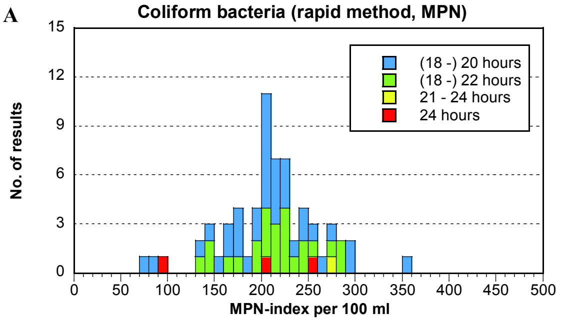
Incubation time	**N**	**A**						**B**						**C**					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total, Rapid meth.	64	64	**204**	13	0	0	0	64	**48**	10	0	0	0	55	**3876**	14	0	3	0
(18 –) 20 hours	36	36	**202**	14	0	0	0	36	**47**	9	0	0	0	32	**3715**	14	0	1	0
(18 –) 22 hours	24	24	**208**	10	0	0	0	24	**50**	12	0	0	0	20	**4177**	13	0	2	0
21 – 24 hours	1	1	**–**	–	0	0	0	1	**–**	–	0	0	0	1	**–**	–	0	0	0
24 hours*	3	3	**177**	–	0	0	0	3	**49**	–	0	0	0	2	**3101**	–	0	0	0

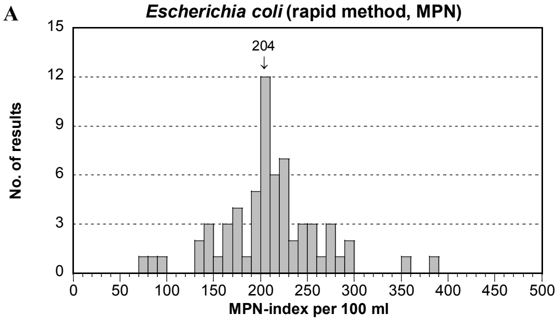
E. coli, Rapid method with MPN

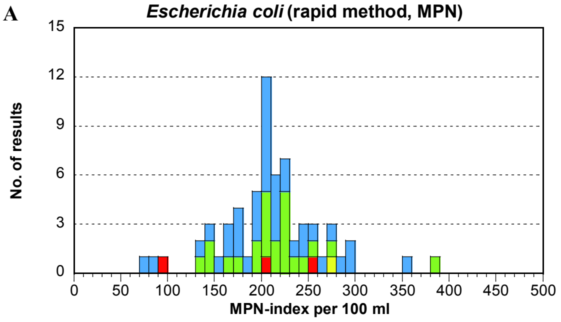
Incubation time	**N**	**A**						**B**						**C**					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total, Rapid meth.	64	64	**204**	13	0	0	0	64	**0**	–	0	–	–	61	**0**	–	1	–	–
(18 –) 20 hours	37	37	**203**	14	0	0	0	37	**0**	–	0	–	–	36	**0**	–	0	–	–
(18 –) 22 hours	23	23	**208**	12	0	0	0	23	**0**	–	0	–	–	21	**0**	–	1	–	–
21 – 24 hours	1	1	**–**	–	0	0	0	1	**0**	–	0	–	–	1	**0**	–	0	–	–
24 hours*	3	3	**177**	–	0	0	0	3	**0**	–	0	–	–	3	**0**	–	0	–	–

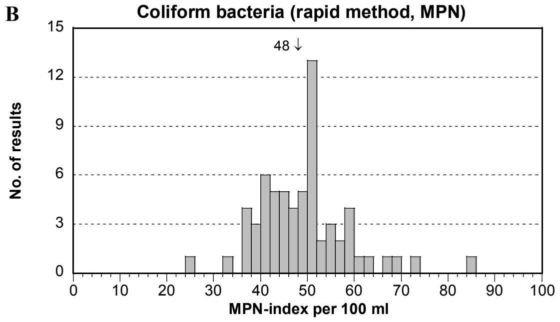
* In one case the method is stated as Colilert 24; mean values are given for comparison despite few results

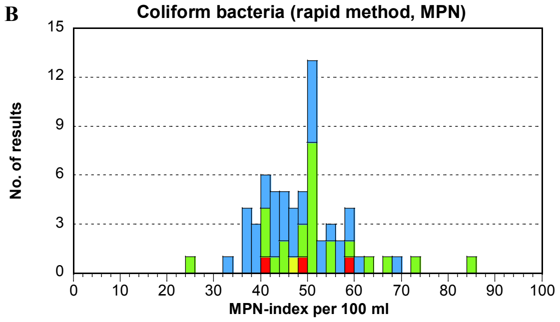

Coliform bacteria (rapid method, MPN)

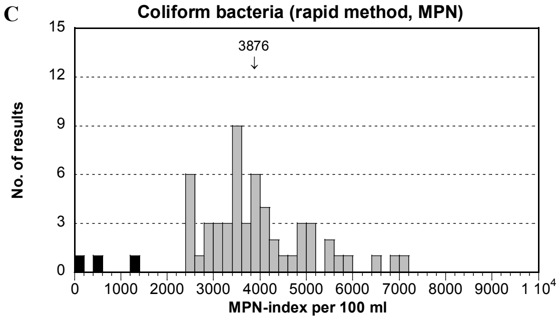

Coliform bacteria (rapid method, MPN)

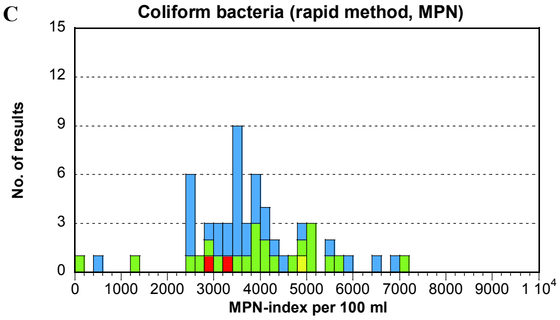

Escherichia coli (rapid method, MPN)


Escherichia coli (rapid method, MPN)


Coliform bacteria (rapid method, MPN)


Coliform bacteria (rapid method, MPN)


Coliform bacteria (rapid method, MPN)


Coliform bacteria (rapid method, MPN)

Mixture A

- The strain of *E. coli* is the only coliform bacterium that grows in the medium and has the enzyme *β*-galactosidase. Therefore, it is detected as coliform bacterium by methods based on this enzyme (ONPG positive) e.g. Colilert®-18/24 Quanti-Tray® where ONPG is a substrate.
- The strain of *E. coli* also has the enzyme *β*-glucuronidase and is detected as *E. coli*.

- The distributions of the results were good and the dispersions (CV) were small in both cases. No outliers or false results were obtained.
- The averages with this rapid method were, as is often the case, somewhat higher than with the MF methods both for coliform bacteria and *E. coli* (compare p. 6).

Mixture B

- Two different coliform bacteria, *C. freundii* and *K. oxytoca*, but no *E. coli* was included.
- The distributions of the results were good and without any peculiarities. The dispersion was small, close to very small, and no deviating results were obtained.
- The average for coliform bacteria was here as in mixture A higher than with the MF methods (compare p. 6), but only a little.

Mixture C

- The mixture contained the three coliform bacteria *E. coli*, *E. cloacae* and *H. alvei*. All of them possess *β*-galactosidase (ONPG positive) and are detected as coliform bacteria. However, that activity is considerably lower for *H. alvei* than for the other two bacteria. To get a positive response for this strain, the trays need to be incubated for 22 hours.
- The distribution of the results for coliform bacteria was in principle good but with a strange peak in its lowest range of the accepted results. That peak is a probable outcome of the interpretation of *H. alvei* as negative due to a too short incubation. The dispersion was small even with this extra peak. Three low outliers were present.
- The strain of *E. coli* possesses *β*-glucuronidase but the activity is so low that the results are usually interpreted as negative. One non-zero result was present but is here in the light of all other results evaluated as false positive.
- The average result for coliform bacteria was also here only marginally higher than it was with the MF methods (compare p. 6).

Presumptive and confirmed *Clostridium perfringens* (MF)

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

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

Due to the hesitation in many countries to use this method the use of a standard still under process (ISO/CD 6461-2:2002-12-20, CD = Committee Draft), based on TSC agar (TSC), was accepted as an alternative by the responsible group under the EU Commission until a finished standard was available. Adjustments in the draft approved during the standardization process have been included in the instructions for proficiency testing rounds, e.g. colour on colonies to be counted.

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

Fourteen out of 58 laboratories have still used one of the older methods that are now replaced by (EN) ISO 14189. Mean values and dispersion are given also for m-CP agar, despite the few results, in order to facilitate comparison both here and with previous PT rounds.

For mixture B m-CP agar gave lower recovery, like in previous rounds, compared to TSC (which is the medium in the two other method references) both for presumptive *C. perfringens* and for *C. perfringens*. For presumptive *C. perfringens* in mixture C only 1 laboratory has used m-CP agar. The histograms show clearly that the results of m-CP agar are mainly in the lower end of the results. Lower results with m-CP agar cannot be taken to be generally valid but have been seen before when the strains of *C. bifermentans* and *C. perfringens* used here have been included.

For the two methods with TSC no difference can be seen in mixture B. In mixture C, however, the older CD version of the standard seemed to give much lower results compared to the final version of the standard.

Mixture A

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

Presumptive Clostridium perfringens MF

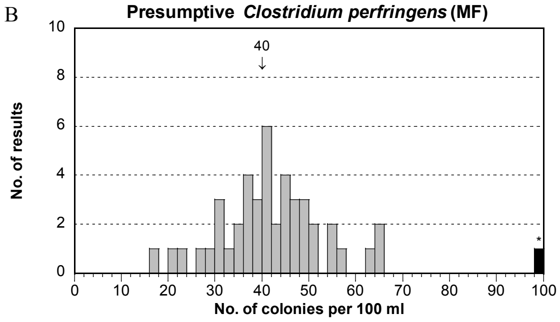
Standard/Method	N[#]	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	58	44	**0**	–	1	–	–	44	**40**	14	0	0	1	38	**1916**	55	4	0	0
(EN) ISO 14189	44	33	**0**	–	1	–	–	33	**42**	13	0	0	1	29	**2295**	51	4	0	0
ISO/CD 6461-2:2002	10	8	**0**	–	0	–	–	8	**39**	11	0	0	0	8	**1110**	56	0	0	0
m-CP agar, EU-direct.	4	3	**0**	–	0	–	–	3	**27***[*]*	–	0	0	0	1	**62***[*]*	–	0	0	0
Other/Unknown	0	0	**–**	–	–	–	–	0	**–**	–	–	–	–	0	**–**	–	–	–	–

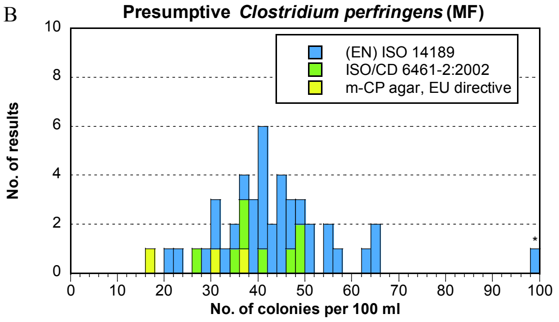
Clostridium perfringens MF

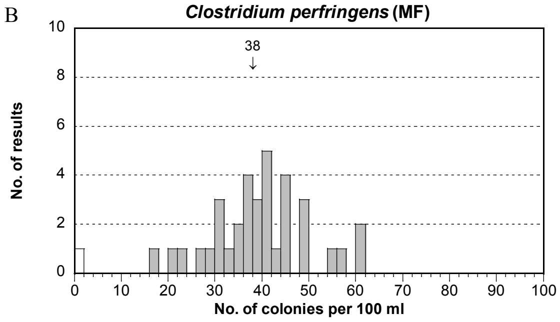
Standard/Method	N[#]	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	58	36	**0**	–	1	–	–	35	**38**	14	1	0	0	31	**0**	–	3	–	–
(EN) ISO 14189	44	25	**0**	–	1	–	–	24	**39**	13	1	0	0	22	**0**	–	2	–	–
ISO/CD 6461-2:2002	10	7	**0**	–	0	–	–	7	**40**	14	0	0	0	6	**0**	–	1	–	–
m-CP agar, EU-direct.	4	4	**0**	–	0	–	–	4	**30***[*]*	–	0	0	0	3	**0**	–	0	–	–
Other/Unknown	0	0	**–**	–	–	–	–	0	**–**	–	–	–	–	0	**–**	–	–	–	–

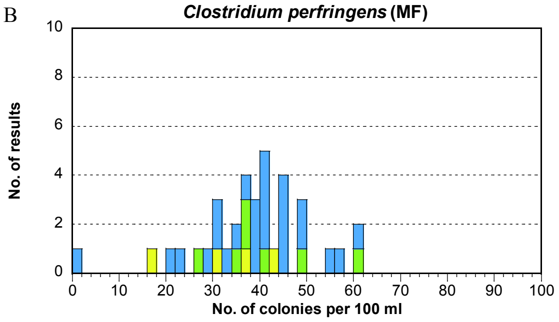
[*] Mean values are given for comparison despite few results

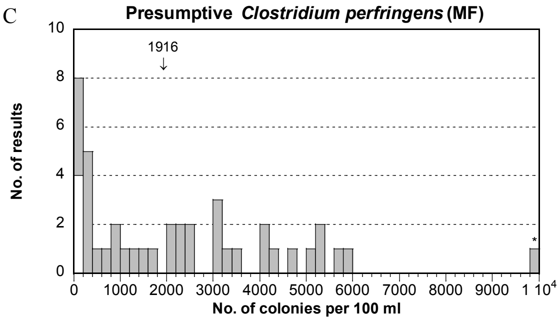
[#] The sum of laboratories that have reported results for presumptive *C. perfringens*, and/or *C. perfringens*

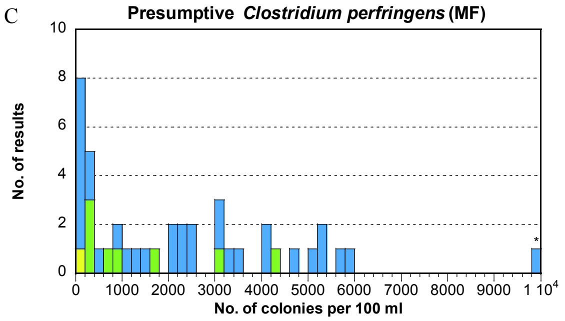












Mixture B

- A strain of *C. perfringens* was included. The colour of the colonies on TSC could vary from pale grey-brown to completely black depending on the condition and reduction potential of the medium.
- One high outlier was present in the presumptive test and 1 false negative result was present for *C. perfringens*.
- The distribution of the results was unusually good for both presumptive and confirmed C. *perfringens*, without the earlier occurring tail of low results. The reason is probably that it this time was only very few results from m-CP agar that earlier have given lower results than TSC. The dispersion (CV) was this time not higher than for other parameters, but was instead small (see p. 30).

Mixture C

- No *C. perfringens* was included but a strain of *C. bifermentans*. The strain appeared on TSC with small, black to almost transparent presumptive colonies. Confirmation reveals that they are not from *C. perfringens*.
- There is no tendency to Poisson distribution of the results as there are many low values. The dispersion (CV) was very large implying that no outliers could be identified. Four zero results were obtained, out of which one was from m-CP agar.
- In the analyses of *C. perfringens* 3 false positive results were present.

Moulds and yeasts (MF)

Out of the 43 laboratories that analysed moulds and yeasts, 32 reported that they used the Swedish standard SS 028192. Besides Sweden it is used in Denmark and also in Finland and Norway under their own national designations SFS 5507 and NS 4716, respectively.

Various names, some appropriate and other probably inappropriate, were reported for the media linked to the use of SS 028192. These are "Cooke Rose Bengal Agar base", "Rose Bengal Agar base", "Rose Bengal Agar", "Rose Bengal Chloramphenicol Agar" (RBC) and "Dichloran Rose Bengal Chloramphenicol Agar" (DRBC). According to the standard, dichloran should not be an ingredient (and thus DRBC should not be used) but instead Rose Bengal and the two stronger inhibitory substances chlortetracycline and chloramphenicol. Both of them are at least used by 17 of the 24 Swedish laboratories. Here is shown what the laboratories have really stated, and a separation is made for those that have used any form of Rose Bengal Agar (RBC Agar) and those stating DRBC in conjunction with SS 028192 or SFS 5507 – or in one case "Standard methods [5] – (DRBC Water).

Two Norwegian laboratories instead used NMKL 98:2005, modified together with DRBC. This comprises the group DRBC Food in the tables. Four Finnish laboratories used "Malt Extract Agar" (ME); one in conjunction with NMKL 98:2005 and the remaining three with other non-water methods. Also a Swedish laboratory has stated ME but in conjunction with SS 028192. These 5 laboratories are placed in the group ME. Three Finnish laboratories using "Oxytetracycline

Glucose Extract Agar" based on other methods/standards are placed in the group OGYE. In several of these groups there are so few results that it is not meaningful to discuss possible differences. But the mean values are still given for comparison.

RBC has given lowest results for moulds and yeasts in mixture A. However, this is not true for moulds in mixture C. In all five cases a selective substance (dichloran, chloramphenicol or streptomycin) has been added to ME, making it selective. This can explain while the ME results are not higher than for the other media.

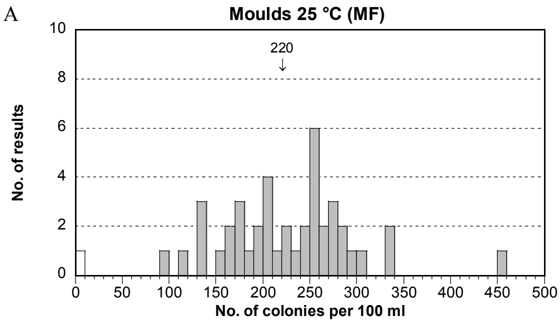
Moulds MF

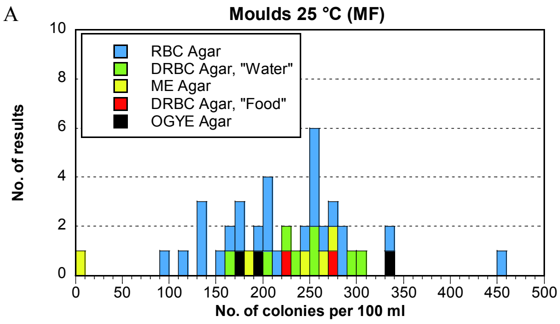
Standard/Method	N	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	43	42	**220**	15	1	0	0	40	**0**	–	3	–	–	41	**288**	9	1	0	0
RBC	25	25	**209**	18	0	0	0	23	**0**	–	2	–	–	25	**290**	10	0	0	0
DRBC Water	8	8	**237**	10	0	0	0	8	**0**	–	0	–	–	7	**305**	5	0	0	0
ME	5	4	**236***	–	1	0	0	4	**0**	–	1	–	–	4	**252***	–	1	0	0
DRBC Food	2	2	**244***	–	0	0	0	2	**0**	–	0	–	–	2	**254***	–	0	0	0
OGYE	3	3	**225***	–	0	0	0	3	**–**	–	0	–	–	3	**306***	–	0	0	0

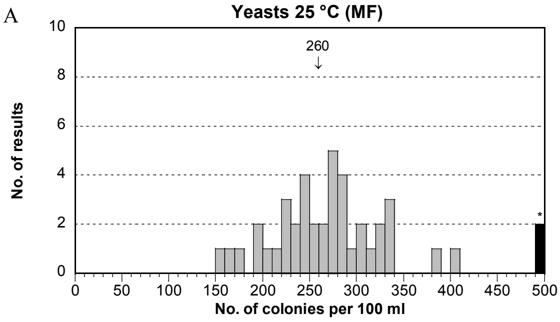
Yeasts MF

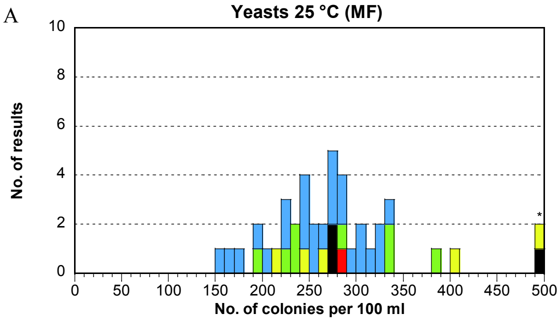
Standard/Method	N	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	42	40	**260**	11	0	0	2	41	**0**	–	1	–	–	39	**0**	–	2	–	–
RBC	25	25	**253**	10	0	0	0	25	**0**	–	0	–	–	24	**0**	–	1	–	–
DRBC A Water	8	8	**271**	12	0	0	0	8	**0**	–	0	–	–	7	**0**	–	0	–	–
ME	5	4	**273***	–	0	0	1	4	**0**	–	1	–	–	4	**0**	–	1	–	–
DRBC Food	1	1	**280***	–	0	0	0	1	**0**	–	0	–	–	1	**0**	–	0	–	–
OGYE	3	2	**270***	–	0	0	1	0	**0**	–	0	–	–	3	**0**	–	0	–	–

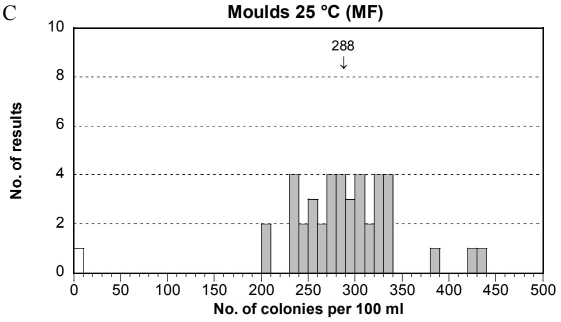
* Mean values are given for comparison despite few results

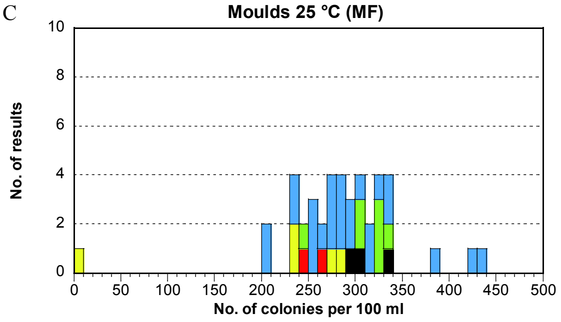












Mixture A

- The mould *Ph. fastigiata* and the yeast *C. glabrata* were included in approximately the same concentrations. No apparent problem could be seen and the distributions of the results were good with small dispersion (CV) for both parameters.
- One false negative result was present for the moulds.
- Two high outliers were present for the yeasts.

Mixture B

- Neither moulds nor yeasts were included. Yet, 3 false positive results were reported for moulds and 1 false positive result for yeasts. In two cases where only 1 and 2 mould colonies were found they can be contamination from the laboratory air. Such results should not be seen as false positive ones.

Mixture C

- No yeasts but the mould *Ph. glomerata* were included. The distribution of the moulds results was relatively good with a small dispersion.
- One false negative mould results was present as well as 2 false positive yeast results.
- The laboratory with the false negative mould result for mixture C reported the false negative result also for mixture A, and in parallel also false positive results for yeast in both mixture B and C. Further, the yeast result for mixture A was an outlier. This laboratory, as the only one, has stated the use of ME with only dichloran added as selective agent. However, this is probably not the cause. Instead the results indicate that that laboratory is unpractised in performing analyses of fungi in water.

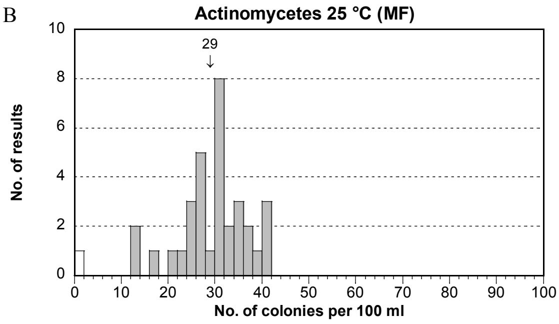
Actinomycetes (MF)

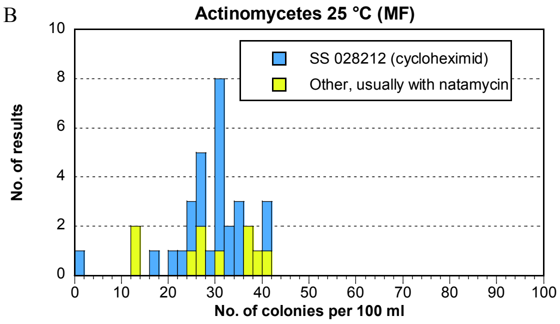
The analysis of actinomycetes is included because it is a prescribed method for drinking water monitoring according to the Swedish regulations. Therefore, it is mainly Swedish laboratories that performed the analysis according to the Swedish standard for actinomycetes in water, SS 028212 (1994). Ten Finnish laboratories that have performed the analysis based on other methods are placed in the group Other. Eight of these have stated that they used natamycin as the selective substance instead of cycloheximide. The remaining 2 laboratories also did not use cycloheximide, but did not specify beyond "Other" what they used. Probably they have used natamycin as well. The base agar medium varies also within the group Other but is in all cases different from Actinomycete Isolation Agar (ACTA) that is the base medium in the Swedish standard.

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

All results

Medium/Standard	N	A						B						C					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total	34	34	**0**	–	0	–	–	33	**29**	13	1	0	0	32	**0**	–	1	–	–
ACTA (SS 028212)	24	24	**0**	–	0	–	–	23	**29**	10	1	0	0	23	**0**	–	1	–	–
Other	10	10	**0**	–	0	–	–	10	**27**	20	0	0	0	9	**0**	–	0	–	–





Mixture A and C

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

Mixtures B

- One actinomycete within the group *Streptomyces* sp. was included. The distribution of the results was good and the average dispersion small.
- One false negative result was present.

Culturable microorganisms 22 °C, 3 days

Seventy-six of the 78 laboratories performing the analysis reported EN ISO 6222:1999 as method, which prescribes the use of Yeast extract Agar. Four laboratories used Plate Count Agar instead and one used R2A agar, but they have simultaneously stated the use of EN ISO 6222:1999. One laboratory used Yeast extract Agar in conjunction with "Standard methods" [5] and stated spread plating instead of pour plating. The majority of the laboratories have claimed counting both bacteria colonies as well as fungal colonies while nine report that they don't count fungi. Three others state that they include yeasts when counting but not moulds.

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

It is difficult to find any consistent method difference. In mixtures A and B, Plate Count Agar seems this time to give lower result than Yeast extract Agar instead of as sometimes higher. However, only 4 results make the difference uncertain. No general difference was seen in relation to magnification. There might be a tendency to results increasing with magnification in mixture C but it is weak. In this round the culturable microorganisms were easy to count in all samples and there were no small colonies present that could be difficult to distinguish. This could explain why there were no clear differences.

The distributions were good for all mixtures and the dispersions were small to very small (see p. 30). Only 2 deviating results were reported.

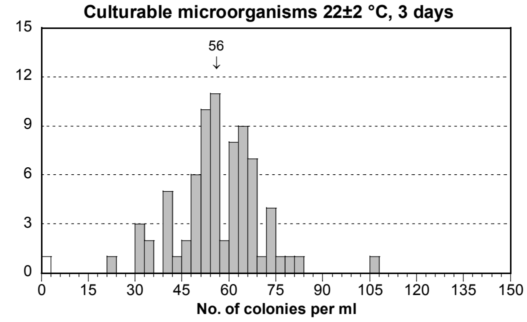
Group of results	**N**	**A**						**B**						**C**					
		n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>	n	**Mv**	CV	F	<	>
Total, all results	78	76	**56**	12	1	0	0	75	**57**	8	0	1	0	76	**45**	10	0	0	0
EN ISO 6222	*76*	*74*	***56***	*12*	*1*	*0*	*0*	*74*	***57***	*8*	*0*	*0*	*0*	*74*	***45***	*10*	*0*	*0*	*0*
Medium																			
Yeast extract Agar	74	70	**56**	12	1	0	0	70	**57**	8	0	0	0	69	**45**	11	0	0	0
Plate Count Agar	5	4	**47***	–	0	0	0	4	**49***	–	0	0	0	4	**46***	–	0	0	0
Other/Unknown	0	0	–	–	–	–	–	0	–	–	–	–	–	1	–	–	0	0	0
Magnification																			
None	18	18	**57**	12	0	0	0	18	**60**	7	0	0	0	18	**42**	8	0	0	0
1,1–4,9×	29	27	**53**	15	1	0	0	27	**54**	8	0	0	0	28	**45**	12	0	0	0
5–11,9×	29	29	**58**	8	0	0	0	29	**57**	8	0	0	0	28	**48**	9	0	0	0
> 12×	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
Other method	2	2	–	–	0	0	0	1	–	–	0	1	0	2	–	–	0	0	0

* Mean values are given for comparison despite few results

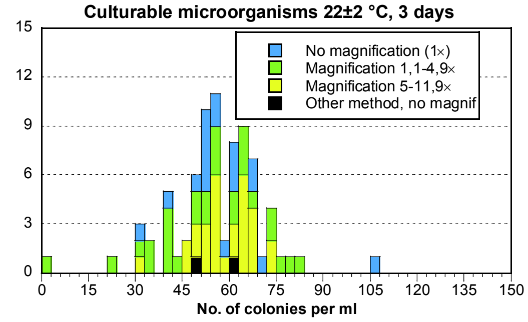
Mixture A

- It is mainly colonies of *S. maltophilia* that are visible but the other bacteria and the yeasts may also appear with individual colonies.
- The distribution of the results was good, but with 1 false negative result.

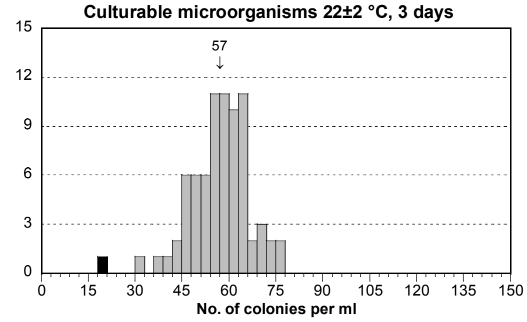
A



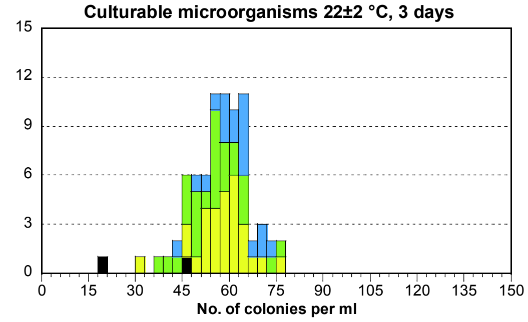
A



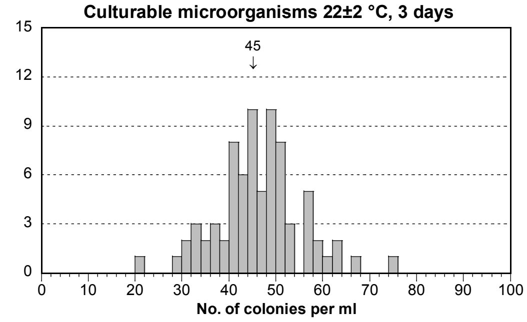
B



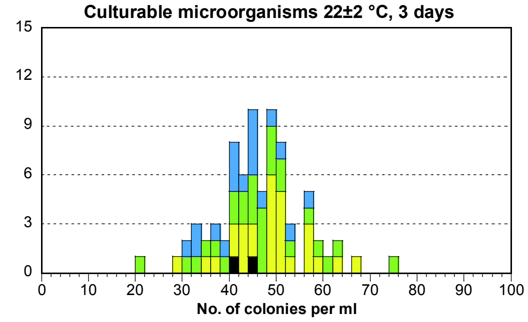
B



C



C



Mixture B

- The colonies mainly consist of the strain of *S. saprophyticus* but individual colonies of the coliform bacteria and the actinomycete may also appear.
- The distribution of the results was good with only 1 low outlier.

Mixture C

- The colonies that appear on the plates are from the three coliform bacteria *E. coli*, *E. cloacae* and *H. alvei* in approximately the same numbers of each.
- The distribution of the results was generally good and without deviating results.

Outcome of the results and laboratory assessment

General information about reported results

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

Base for assessment of the performance

The laboratories are not grouped or ranked in relation to their performances. The performance can broadly be assessed by the numbers of false results and outliers given beneath the box plots.

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

Mixed up results and other practical errors

A number of laboratories have several deviating results. When whole samples seem to have been mixed up, the corresponding sample numbers are hatched in annex A. This time no laboratory seems to have mixed up vials. One laboratory seems to have mixed up two results for presumptive *C. perfringens*. Four laboratories seem to have performed incorrect calculations from their colony readings to the final concentrations.

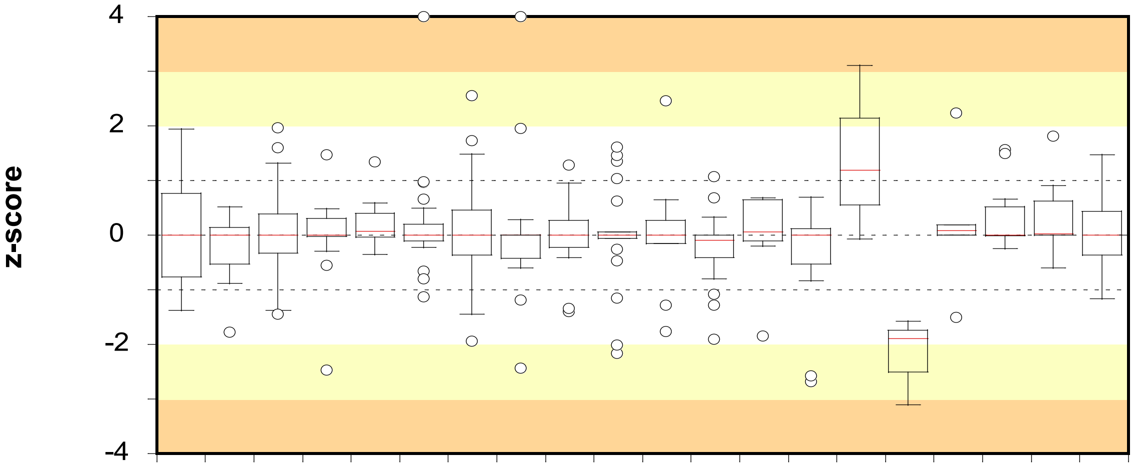
Z-scores, box plots and deviating results for each laboratory

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

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

Box plots and numbers of deviating results for each participating laboratory

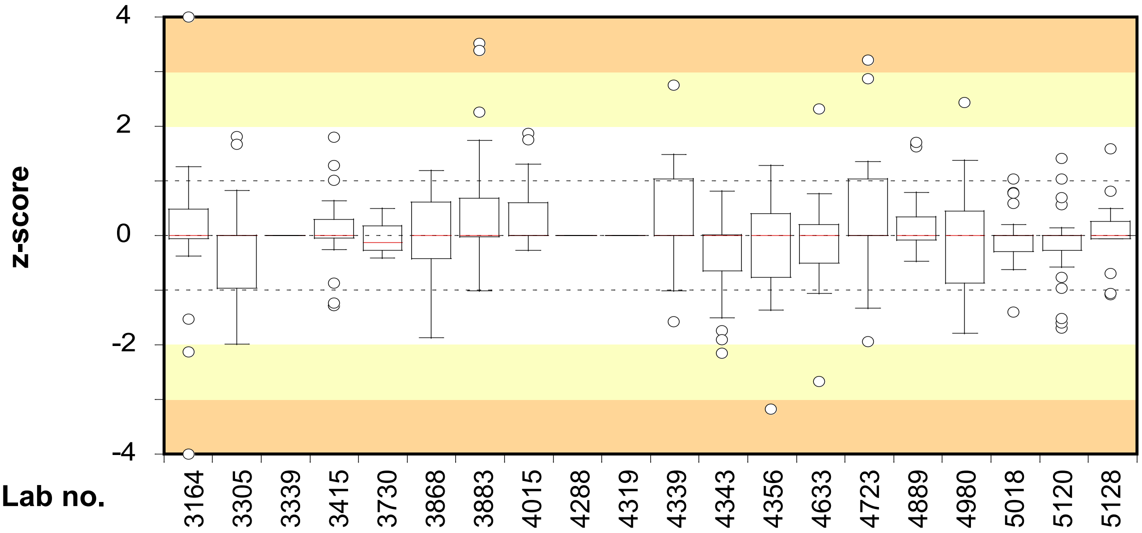
- *z-scores are calculated from the formula z = (x – mv) / s (see annex A).*
- *A correct result "zero" will get z = 0 when there is no target organism present.*
- *False results do not generate z-scores and are not included in ‘No. of results’.*
- *The outliers are included in the plots after recalculation to standardised values with the same standard deviation (s) as the rest of the results.*
- *z-scores > +4 and < –4 have in the plots been set to +4 and –4, respectively.*
- *The numbers of false positives and false negatives are given in the table under the plots together with the numbers of outliers.*
- *The horizontal red line in each box indicates the median for the laboratory.*
- *The box includes 25% of the results above and below the median. The lines protruding from the box and/or the circles embrace the remaining 50% of the results, false results excluded.*
- *A circle is shown when a result is to a certain degree deviating* from the rest.*
- *The background is divided into coloured fields to simplify localization of the laboratory results.*



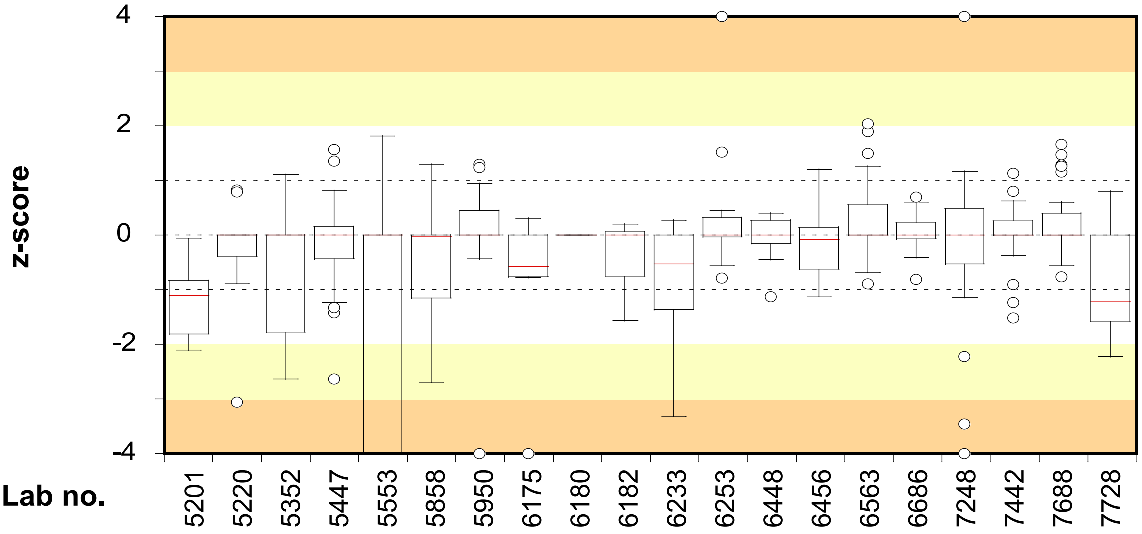
Lab no.	1131	1237	1545	1594	1611	1753	1868	1970	2050	2386	2637	2704	2745	2944	3055	3076	3145	3155	3159	3162
No. of results	12	15	30	24	15	27	27	21	27	21	9	20	8	12	3	3	6	14	12	21
False positive	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
False negative	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-	-

---

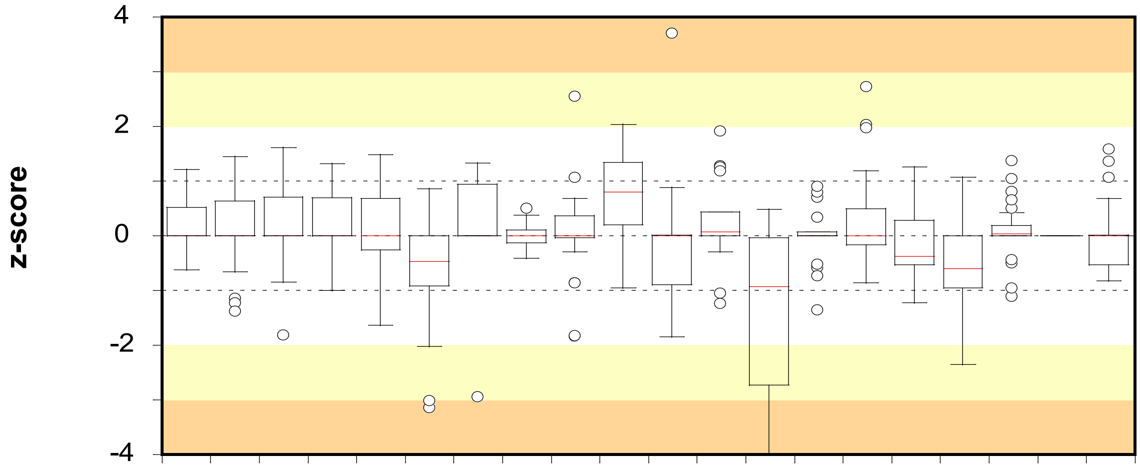
* < [smallest value of the box - 1.5 × (largest value of the box - smallest value of the box)] *or* > [largest value of the box + 1.5 × (largest value of the box - smallest value of the box)]



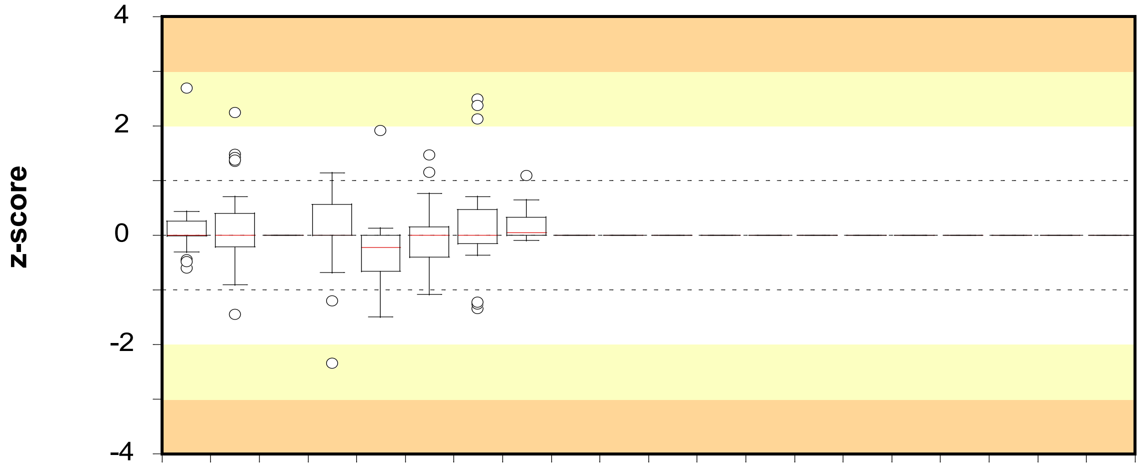
Lab no.	3164	3305	3339	3415	3730	3868	3883	4015	4288	4319	4339	4343	4356	4633	4723	4889	4980	5018	5120	5128
No. of results	19	26	-	26	3	19	27	21	-	-	29	21	17	24	21	16	15	29	26	14
False positive	2	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	1
False negative	2	1	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Low outliers	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
High outliers	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-



Lab no.	5201	5220	5352	5447	5553	5858	5950	6175	6180	6182	6233	6253	6448	6456	6563	6686	7248	7442	7688	7728
No. of results	6	10	21	24	7	13	27	8	-	7	15	15	8	15	26	11	29	24	30	9
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	1	-	-
False negative	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-
Low outliers	-	-	-	-	3	-	1	1	-	-	-	-	-	-	-	-	1	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-



Lab no.	7876	7930	7962	7968	8019	8068	8252	8260	8329	8380	8435	8569	8626	8628	8663	8742	8751	8766	8840	8862
No. of results	27	27	20	27	23	23	9	15	26	14	11	19	9	17	21	9	9	27	-	29
False positive	-	-	-	-	-	1	-	-	1	1	-	1	-	-	-	-	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-



Lab no.	8898	8955	8998	9436	9524	9736	9899	9903
No. of results	27	26	-	27	21	19	27	21
False positive	-	1	-	-	-	1	-	-
False negative	-	-	-	-	-	1	-	-
Low outliers	-	-	-	-	-	-	-	-
High outliers	-	-	-	-	-	-	-	-

Test material, quality controls and processing of data

Description of the test material

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

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

Table 2 *Microorganisms present in the mixtures*

Mixture [1]	**Microorganisms**	**Strain collection no.**		**cfu/100 ml** [2]
		SLV (own)	Reference [3]	
A	*Escherichia coli*	165	CCUG 43600	220
	Aeromonas hydrophila	533	CCUG 48892	280
	Candida glabrata	052	CBS typed	270
	Phialophora fastigiata	504	CBS typed	220
	Stenotrophomonas maltophilia	041	–	71 *
B	*Citrobacter freundii*	091	CCUG 43597	34
	Klebsiella oxytoca	553	From water	29
	Clostridium perfringens	442	CCUG 43593	38
	Streptomyces sp.	548	From water	34
	Staphylococcus saprophyticus	013	CCUG 45100	57 *
C	*Escherichia coli*	295	From water	2100
	Enterobacter cloacae	187	CCUG 43599	1700
	Hafnia alvei	015	CCUG 45642	1300
	Clostridium bifermentans	009	CCUG 43592	320
	Phoma glomerata	543	CBS 119226	290

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

3 Origin or typing collection no.; ATCC: American Type Culture Collection; CCUG: Culture Collection University of Gothenburg, Sweden; CBS: Centraalbureau vor Schimmelcultures, Utrecht, Holland; – or "From water" indicate a strain from our own culture collection

Quality control of the test material

It is essential to have a homogeneous mixture and a uniform volume in all vials in order to allow comparison of all freeze-dried samples derived from one mixture. The volume was checked by weighing 2 to 3% of the number of vials produced, of the mixtures. The largest differences between vials were 8, 4 and 4 mg in mixture A, B and C respectively. The largest accepted difference is 15 mg (3%).

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

Analysis parameter	**Mixture**								
Method standard for analysis	**A**[1]			**B**[1]			**C**[2]		
	cfu	**I_2**	**T**	**cfu**	**I_2**	**T**	**cfu**	**I_2**	**T**
Coliform bacteria (MF) *m-Endo Agar LES according to SS 028167*	22[a]	0.6	1.4	63	0.3	1.2	39[b]	1.9	1.6
Suspected thermotolerant colif. bact. (MF) *m-FC Agar, 44 °C according to SS 028167*	20[a]	0.7	1.4	0	–	–	20[c]	0.7	1.5
Escherichia coli (MF) *m-Endo Agar LES according to SS 028167*	22[a]	0.6	1.4	0	–	–	21[b]	0.8	1.5
Presumptive *Clostridium perfringens* (MF) *TSC Agar according to ISO/CD 6461-2:2002*	–	–	–	38	0.6	1.3	32[a]	2.8	1.9
Moulds (MF) *Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192*	22[a]	0.9	1.5	–	–	–	29[a]	0.8	1.4
Yeasts (MF) *Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192*	27[a]	0.5	1.3	–	–	–	–	–	–
Actinomycetes (MF) *Actinomycete Isolation Agar with cycloheximide according to SS 028212*	–	–	–	34	1.7	1.6	–	–	–
Culturable microorg., 3d 22 °C (pour plate) *Yeast extract Agar according to SS-EN ISO 6222:1999*	79	0.9	1.2	58	1.1	1.3	49	1.3	1.4

1 10 vials analysed in duplicate, normally100 ml for MF and 1 ml for pour plate, analysed 21, 19 and 14 weeks ahead of the testing round for the mixtures A, B and C, respectively

a Determined for the volume 10 ml

b Determined for the volume 1 ml

c Determined for the volume 1 ml; only *E. coli* but not *E. cloacae* was included

– No target organism and thus no analysis

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

Processing of numerical results

Most histograms have "tails" in either or both directions, due to values that do not belong to a normal distribution. Calculations are performed after square root transformations of the results that give better normal distributions by decreasing the significance of the high end "tails". Very deviating values are still present in most analyses and are identified as outliers (black bars). False negative results are presented with white bars in the histograms.

Outliers are identified by use of Grubbs' test according to a modification by Kelly [3]. A level of 1% is set as the risk to incorrectly assess a result as being an outlier. Although the method is objective, there is a prerequisite that the results are normally distributed in order to obtain correct outliers at the 1% level. A zero result that is a low outlier is considered a false negative result. In special situations, e.g. when many zero results are reported and in some borderline cases, a few subjective adjustments are made in order to set the right limits based on the knowledge of the mixture's contents. False results and outliers are not included in the calculations of mean values and measures of distribution.

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

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

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

References

1. Anonymous 2018. Scheme protocol, Microbiology, Drinking water & Food, 5th 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/
6. Anonymous 2015. Commission Directive (EU) 2015/1787 of 6 October 2015 amending Annexes II and III to Council Directive 98/83/EC on the quality of water intended for human consumption. Official Journal of the European Union. 7.10.2015, L 260/6-17 *(national translations available)*.

Annex A *Results of the participants, cfu/100 ml (see also the note). Susp. = suspected on membrane filter before confirmation. Results given as <1, <2, <10 and <100 are treated as zero. The fields with other results given as < 'value' and results given as > 'value' are* **yellow***, and those results are not included in calculations or evaluations, 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*

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			*E. coli* (MF)			Coliform bacteria ("rapid" MPN)			*E. coli* ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	248	38	3448	248	0	0
1237	1	3	2	-	-	-	180	38	2000	-	-	-	180	<1	**<1**	220	40	**>2420**	220	<1	<1
1545	3	2	1	430	32	5800	160	32	5800	160	0	4600	160	0	3500	192	45	5100	192	0	0
1594	3	2	1	445	51	4000	190	51	4000	170	0	2200	190	0	2200	230	46	3300	230	0	0
1611	3	2	1	500	62	3900	206	62	3900	150	0	2700	206	0	2730	186	51	3683	186	0	0
1753	3	2	1	361	57	3500	178	57	3500	-	-	-	178	0	**0**	220	42	5000	220	0	0
1868	3	1	2	209	64	2564	209	64	2564	-	-	-	209	0	1477	162	55	2551	162	0	0
1970	3	1	2	520	39	3500	**520**	39	3500	520	0	3500	**520**	0	2000	-	-	-	-	-	-
2050	2	1	3	-	-	-	170	49	4100	-	-	-	170	0	2100	137	49	4970	137	0	0
2386	1	2	3	250	58	4200	140	58	4200	190	0	2300	190	0	2300	201	62	3600	201	<1	<1
2637	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	240	48	7000	220	<1	<1
2704	3	1	2	-	-	-	170	26	2800	-	-	-	170	0	2800	222	59	3440	222	<1	<1
2745	2	1	3	310	43	4200	220	43	4200	220	**43**	4200	220	**26**	2320	-	-	-	-	-	-
2944	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	87	47.8	4530	87	<1	<1
3055	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	214	72	2420	214	0	0
3155	3	2	1	332	43	-	177	43	-	140	<1	-	177	<1	-	295	48	-	295	<1	-
3159	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	207	53.1	3840	207	<1	<1
3162	1	3	2	-	-	-	-	-	-	-	-	-	-	-	-	249	42	3076	249	0	0
3164	1	2	3	-	-	-	210	61	**240**	-	-	-	210	0	**0**	260	47	2400	260	0	0
3305	3	1	2	-	-	-	110	28	5600	-	-	-	110	<1	2900	200	33	5900	200	<1	<1
3339	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3415	2	1	3	380	48	3350	210	48	3350	-	-	-	210	0	**0**	142	67	2613	142	0	0
3730	2	1	3	440	45	2200	-	-	-	260	0	2100	-	-	-	-	-	-	-	-	-
3868	2	1	3	400	60	-	150	60	-	110	0	-	110	0	-	250	48	-	250	0	-
3883	3	1	2	630	75	5100	**378**	75	5100	-	-	-	**378**	0	2300	210	50	5480	210	0	0
4015	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	190	50	5000	190	0	0
4288	1	2	3	24	52	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4339	2	1	3	540	61	3300	260	61	3300	140	0	3200	260	0	**0**	285	40	4600	385	0	0
4343	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	205	50	2420	205	0	0
4356	1	2	3	400	50	3300	250	50	3300	250	0	2000	250	**9**	2700	200	50	**1200**	200	0	**440**
4633	1	2	3	-	-	-	194	53	4100	145	0	1855	194	0	4100	152	43	3400	152	0	0
4723	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	140	84	5475	140	0	0
4889	1	2	3	-	-	-	270	47	3100	-	-	-	270	0	**0**	200	56	**>2400**	200	0	0
4980	2	3	1	-	-	-	-	-	-	-	-	-	-	-	-	144.5	42.9	6970	144.5	<1	<1
5018	1	2	3	450	58	3100	225	58	3100	-	-	-	225	0	**0**	179	50	3440	179	0	0
5120	3	1	2	-	-	-	120	52	3700	210	0	0	120	0	**0**	190	41	2900	190	0	0
5128	2	3	1	-	-	-	-	-	-	-	-	-	140	<1	1900	201	50	4800	201	<1	<1
5201	2	3	1	-	-	-	185	-	1760	-	-	-	112	-	1760	-	-	-	-	-	-
5220	3	1	2	-	-	-	175	40	1150	-	-	-	175	0	**0**	250	40	**> 2419**	250	0	0
5352	3	2	1	-	-	-	117	20	1780	-	-	-	90	<1	1120	-	-	-	-	-	-
5447	3	1	2	-	-	-	130	20	5300	-	-	-	130	0	3300	-	-	-	-	-	-
5553	2	1	3	-	-	-	**0**	**10**	**380**	-	-	-	**0**	0	**240**	-	-	-	-	-	-
5858	3	2	1	-	-	-	205	33	4975	-	-	-	132	<1	**<1**	-	-	-	-	-	-
5950	1	2	3	227	46	3900	227	46	3900	164	<1	2200	227	<1	2500	278	44	**483**	278	<1	<1
6175	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	165	43	**>200**	165	0	0
6180	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6182	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	130	50	3937	130	0	0
6233	2	1	3	300	45	3500	200	45	3500	-	-	-	200	0	2000	172	36	2480	172	0	0
6253	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	200	49	3300	200	0	0
6448	3	2	1	4200	50	3900	200	50	3900	-	-	-	200	0	**0**	-	-	-	-	-	-
6456	1	3	2	-	-	-	160	37	2800	-	-	-	160	0	2700	200	38	3400	200	0	0
6563	2	3	1	436	61	3700	175	61	3700	-	-	-	175	0	2960	228	68	4267	198	0	0
6686	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	200.5	40.6	3440	200.5	<1	<1
7248	3	1	2	380	531	3850	210	**531**	3850	240	0	2100	240	0	2000	270	54	**41**	270	0	0
7442	3	2	1	433	41	3846	217	41	3846	-	-	-	217	0	**0**	216	37	3995	216	0	0
7688	1	3	2	540	49	4800	250	49	4800	-	-	-	250	0	3400	190	61	3500	190	0	0
7728	3	1	2	-	-	-	135	37	2000	-	-	-	135	0	1100	-	-	-	-	-	-
7876	1	2	3	409	54	3400	170	54	3400	109	<1	1864	170	<1	2300	210	55	4106	201	<1	<1
7930	1	2	3	220	38	2500	220	38	2500	-	-	-	220	0	1600	240	50	5600	240	0	0
7962	1	2	3	490	36	4600	170	36	4600	124	0	3400	170	0	**0**	291	45	4110	291	0	0
7968	1	3	2	490	45	5000	240	45	5000	147	0	2400	240	0	2400	210	39	3448	210	0	0
8019	1	3	2	430	42	3600	260	42	3600	180	<1	1800	260	<1	**<1**	275	46	4884	275	<1	<1
8068	1	3	2	-	-	-	153	42	3100	-	-	-	153	**21**	2000	72	36	2400	72	0	0
8252	~~2~~	~~1~~	3	51	61	50	-	-	-	-	-	-	-	-	-	280	24	4100	280	<1	<1
8260	1	2	3	345	41	3270	195	41	3270	-	-	-	195	<1	2370	-	-	-	-	-	-
Mean							**188**	**45**	**3552**				**187**	**0**	**2330**	**204**	**48**	**3876**	**204**	**0**	**0**
CV (%)							12	13	14				12	-	14	13	10	14	14	-	-

the summarizing calculated results at the end of the table. The mean value (Mean) is the square of the mean value for the square root transformed results (mv). The coefficient of variation (CV) is the standard deviation (s) in percentage of the mean value for the square root transformed results. As means to calculate the z-values of your own, the appropriate values of mv and s are given at the end of the table. The x-values of a laboratory are obtained as the square roots of each reported result, respectively.
z = (x - mv) / s. $u_{rel,mv}$ is the relative standard uncertainty of mv in per cent. For calculation see the scheme protocol [1]; also briefly described in the text.

Presumptive *C. perfringens* (MF)			*Clostridium perfringens* (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22±2 °C, 3 days $^{\#}$			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0	65	240	-	-	-	-	-	-	-	-	-	-	-	-	64	45	53	**1131**
-	-	-	**88**	44	<1	-	-	-	-	-	-	-	-	-	50	61	40	**1237**
0	26	650	0	26	0	290	0	330	190	0	0	0	31	0	68	69	49	**1545**
-	-	-	-	-	-	330	0	290	270	0	0	0	13	0	52	61	45	**1594**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	63	56	46	**1611**
0	**120**	2200	0	-	-	210	0	250	250	0	0	0	29	0	65	47	50	**1753**
0	62	2036	-	-	-	173	0	427	164	0	0	0	34	0	60	61	49	**1868**
0	34	1600	0	34	0	200	0	300	380	0	0	-	-	-	41	37	48	**1970**
0	43	4000	-	-	-	200	0	270	310	0	0	0	30	0	54	65	58	**2050**
0	20	1050	0	20	0	-	-	-	-	-	-	-	-	-	77	72	45	**2386**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	42	44	**2637**
<1	36	**<1**	<1	36	<1	-	-	-	-	-	-	-	-	-	40	55	36	**2704**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57	57	30	**2745**
-	-	-	<1	41	<1	-	-	-	-	-	-	-	-	-	53	63	38	**2944**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	105	56	57	**3055**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	41	32	**3076**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3145**
<1	44	-	<1	44	-	-	-	-	-	-	-	-	-	-	65	57	-	**3155**
-	-	-	<1	60	<1	-	-	-	-	-	-	-	-	-	66	65	40	**3159**
0	48	350	-	-	-	250	0	270	200	0	0	0	25	0	65	56	60	**3162**
-	-	-	-	-	-	**0**	0	**0**	**600**	**65**	**450**	0	31	0	31	58	42	**3164**
-	-	-	<1	**<1**	<1	240	<1	230	210	<1	<1	<1	26	<1	34	59	46	**3305**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**3339**
0	40	5600	0	40	0	165	0	320	280	0	0	-	-	-	55	66	43	**3415**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54	53	50	**3730**
0	30	-	0	30	-	250	0	-	330	0	-	0	38	-	**0**	46	-	**3868**
0	30	5200	-	-	-	190	0	380	270	0	0	0	26	0	53	49	41	**3883**
0	64	3400	-	-	-	207	0	332	338	0	0	0	30	0	54	61	63	**4015**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**4288**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**4319**
0	39	35	0	39	0	250	0	240	330	0	0	0	37	0	73	60	47	**4339**
0	50	3	-	-	-	136	0	282	155	0	0	0	16	0	57	51	49	**4343**
3100	30	**0**	0	30	0	-	-	-	-	-	-	-	-	-	39	50	51	**4356**
-	-	-	-	-	-	236	0	327	227	0	0	0	12	0	56	48	49	**4633**
0	47	4727	-	-	-	455	0	200	273	0	0	0	26	0	46	68	57	**4723**
-	-	-	0	42	0	-	-	-	-	-	-	-	-	-	81	54	42	**4889**
<1	23	5800	<1	23	<1	-	-	-	-	-	-	-	-	-	52	65	59	**4980**
0	38	98	0	38	0	180	0	270	260	0	0	0	30	0	52	55	51	**5018**
-	-	-	0	37	0	130	0	260	300	0	0	0	37	0	53	70	44	**5120**
-	-	-	<1	28	**4700**	-	-	-	-	-	-	-	-	-	79	59	50	**5128**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	38	**5201**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**5220**
<1	36	280	-	-	-	300	<1	300	230	<1	<1	<1	30	<1	48	61	28	**5352**
0	41	4000	0	41	0	260	0	230	240	0	0	0	25	0	55	64	40	**5447**
-	-	-	0	60	0	-	-	-	-	-	-	-	-	-	-	-	-	**5553**
<1	16	**>300**	<1	16	<1	-	-	-	-	-	-	-	-	-	51	49	45	**5858**
<1	45	2464	-	-	-	255	<1	336	245	<1	<1	<1	30	<1	66	55	44	**5950**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	60	**20**	40	**6175**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**6180**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	46	**6182**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	45	20	**6233**
-	-	-	-	-	-	170	0	300	**3300**	0	0	-	-	-	62	71	49	**6253**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	47	44	**6448**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73	63	48	**6456**
0	46	3282	-	-	-	164	**2**	255	282	0	0	0	41	0	55	63	66	**6563**
<1	40	**<1**	-	-	-	-	-	-	-	-	-	-	-	-	62	62	52	**6686**
0	41	395	0	41	**395**	150	0	235	300	0	0	0	22	0	30	30	50	**7248**
0	54	481	-	-	-	130	**1**	273	282	0	0	0	**0**	0	67	59	42	**7442**
0	45	920	0	45	0	240	0	300	220	0	0	0	26	0	61	53	62	**7688**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62	64	32	**7728**
<1	55	5200	-	-	-	282	<1	318	227	<1	<1	<1	32	<1	61	64	48	**7876**
0	45	5000	0	45	0	280	0	280	190	0	0	-	-	-	74	57	48	**7930**
-	-	-	-	-	-	270	0	290	170	0	0	-	-	-	69	72	50	**7962**
0	49	940	0	49	0	190	0	330	270	0	0	-	-	-	53	63	52	**7968**
<1	33	3100	<1	33	<1	220	<1	245	-	-	-	-	-	-	50	43	56	**8019**
-	-	-	0	48	0	220	0	320	230	0	0	-	-	-	32	48	40	**8068**
-	-	-	<1	49	<1	-	-	-	-	-	-	-	-	-	-	-	-	**8252**
<1	36	3130	<1	36	<1	-	-	-	-	-	-	-	-	-	61	57	45	**8260**
0	**40**	**1916**	**0**	**38**	**0**	**220**	**0**	**288**	**260**	**0**	**0**	**0**	**29**	**0**	**56**	**57**	**45**	**Mean**
-	14	55	-	14	-	15	-	9	11	-	-	-	13	-	12	8	10	**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	A	B	C
8329	2	1	3	193	42	4100	193	42	4100	-	-	-	193	0	1800	225	59	3005	225	0	0
8380	2	3	1	510	62	3900	290	62	3900	-	-	-	290	**24**	3500	250	57	4100	250	0	0
8435	3	1	2	-	-	-	**390**	30	2600	109	0	2000	230	0	**0**	-	-	-	-	-	-
8569	2	3	1	250	50	4000	250	50	4000	-	-	-	250	0	**0**	228	50	2827	228	0	0
8626	1	2	3	430	46	4500	86	**9**	4050	43	0	2250	**43**	0	2250	-	-	-	-	-	-
8628	3	1	2	-	-	-	190	31	3000	110	0	1600	190	0	**0**	-	-	-	-	-	-
8663	3	2	1	490	60	3000	290	60	3000	150	0	2400	290	0	1800	200	44	3700	200	0	0
8742	2	3	1	-	-	-	165	41	2600	-	-	-	165	0	1600	-	-	-	-	-	-
8751	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	99	59	2909	99	<1	<1
8766	1	2	3	315	46	3073	190	46	3073	138	0	2380	190	0	3073	210	44	4350	210	0	0
8840	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8862	2	3	1	273	45	3800	155	45	3800	-	-	-	155	0	**0**	163	59	3130	163	0	0
8898	1	2	3	427	45	3818	200	45	3818	-	-	-	200	0	2364	222	51	3870	222	0	0
8955	2	3	1	-	-	-	150	50	5200	110	0	2800	150	0	3300	170	52	3900	170	0	0
8998	2	1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9436	3	1	2	382	48	4300	218	48	4300	209	0	3300	218	0	2580	235	51	3440	235	0	0
9524	3	1	2	310	39	2900	160	39	2900	-	-	-	160	<1	1900	211	36	3200	211	<1	<1
9736	3	1	2	-	-	-	-	-	-	-	-	-	-	-	-	170	41	3973	170	0	0
9899	3	2	1	133	52	4300	133	52	4300	-	-	-	133	0	2100	357	50	6532	357	0	0
9903	1	3	2	411	47	3833	203	47	3833	189	0	3370	189	0	2290	-	-	-	-	-	-

	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
n	43	43	41	61	60	59	27	27	25	62	61	60	64	64	58	64	64	62
Min	24	32	33	0	9	240	43	0	0	0	0	0	72	24	41	72	0	0
Max	4200	531	5800	520	531	5800	520	43	4600	520	26	4100	357	84	7000	385	0	440
Median	400	48	3818	190	46	3700	150	0	2300	190	0	2300	208.5	48.5	3700	203	0	0
Mean				**188**	**45**	**3552**				**187**	**0**	**2330**	**204**	**48**	**3876**	**204**	**0**	**0**
CV (%)				12	13	14				12	-	14	13	10	14	14	-	-
False positive				0	0	0				0	4	0	0	0	0	0	0	1
False negative				1	0	0				1	0	18*	0	0	0	0	0	0
Outliers, low				0	2	2				1	0	1	0	0	3	0	0	0
Outliers, high				3	1	0				2	0	0	0	0	0	0	0	0
Low limit OK	24	32	33	86	20	1150	43	0	0	90	0	1100	72	24	2400	72	0	0
High limit OK	4200	531	5800	290	75	5800	520	0	4600	290	0	4100	357	84	7000	385	0	0

	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
mv *(√Mean)*				13.712	6.727	59.598				13.670	0.000	48.274	14.286	6.935	62.258	14.297	0.000	0.000
s *(CV*mv/100)*				1.628	0.856	8.410				1.701	0.000	6.797	1.848	0.693	8.689	1.931	0.000	0.000
$u_{rel.mv}$ **(%)** $(100*s/\sqrt{n_{mv}})$				1.6	1.7	1.9				1.6		2.2	1.6	1.2	1.9	1.7		
x *(√Result)*																		
z *([x-mv]/s)*																		

\# *cfu/ml*

* The majority of the 18 resultats is not reckoned as false negative results but as accepted zero results; see the text

Presumptive *C. perfringens* (MF)			***Clostridium perfringens* (MF)**			**Moulds (MF)**			**Yeasts (MF)**			**Actinomycetes (MF)**			**Total plate count 22±2 °C, 3 days $^{\#}$**			**Lab no.**
A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	
0	40	11100	-	-	-	114	0	205	250	0	**132**	0	34	0	63	60	45	**8329**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	68	59	37	**8380**
-	-	-	0	35	0	-	-	-	-	-	-	-	-	-	56	50	30	**8435**
0	40	200	0	40	**200**	-	-	-	-	-	-	-	-	-	52	75	57	**8569**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	44	46	45	**8626**
-	-	-	0	31	0	270	0	263	280	0	0	-	-	-	67	65	46	**8628**
0	38	3100	0	38	0	-	-	-	-	-	-	-	-	-	64	54	74	**8663**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	74	60	48	**8742**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	55	40	**8751**
0	50	2000	-	-	-	255	0	236	264	0	0	0	40	0	65	59	37	**8766**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**8840**
0	57	2300	0	57	0	173	0	255	273	0	0	0	34	0	56	52	42	**8862**
0	34	1364	-	-	-	200	0	436	245	0	0	0	32	0	50	59	41	**8898**
-	-	-	0	55	0	270	**280**	280	400	0	0	0	40	0	53	53	33	**8955**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**8998**
0	47	4200	-	-	-	91	0	318	327	0	0	0	25	0	47	55	35	**9436**
<1	36	62	<1	36	<1	-	-	-	-	-	-	-	-	-	55	75	35	**9524**
0	43	**0**	-	-	-	330	0	327	328	0	0	0	21	**1182**	66	53	42	**9736**
0	28	1419	-	-	-	260	0	290	243	0	0	0	26	0	55	60	51	**9899**
0	48	2417	-	-	-	250	0	283	295	0	0	0	31	0	63	56	56	**9903**

45	45	42	37	36	34	43	43	42	42	42	41	34	34	33	77	76	76	**n**
0	16	0	0	0	0	0	0	0	155	0	0	0	0	0	0	20	20	**Min**
3100	120	11100	88	60	4700	455	280	436	3300	65	450	0	41	1182	105	75	74	**Max**
0	40.5	2118	0	39	0	228	0	283	267	0	0	0	30	0	55.5	57	45.5	**Median**
0	**40**	**1916**	**0**	**38**	**0**	**220**	**0**	**288**	**260**	**0**	**0**	**0**	**29**	**0**	**56**	**57**	**45**	**Mean**
-	14	55	-	14	-	15	-	9	11	-	-	-	13	-	12	8	10	**CV (%)**
1	0	0	1	0	3	0	3	0	0	1	2	0	0	1	0	0	0	**False pos.**
0	0	4	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0	**False neg.**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	**Outliers <**
0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	**Outliers >**
0	16	3	0	16	0	91	0	200	155	0	0	0	12	0	22	30	20	**Low limit**
0	65	11100	0	60	0	455	0	436	400	0	0	0	41	0	105	75	74	**High limit**

0.000	6.359	43.777	0.000	6.192	0.000	14.821	0.000	16.964	16.135	0.000	0.000	0.000	5.349	0.000	7.468	7.526	6.736	**mv**
0.000	0.874	24.116	0.000	0.855	0.000	2.263	0.000	1.452	1.715	0.000	0.000	0.000	0.707	0.000	0.895	0.593	0.683	**s**
	2.1	8.9		2.3		2.4		1.3	1.7				2.3		1.4	0.9	1.2	**$u_{rel.mv}$ *(%)***
																		x
																		z

Annex B *Z-scores calculated from the laboratory results. Susp. = Suspected on the membrane filters before confirmation.* $z = (x - mv) / s$. *Z-scores are calculated also for outliers (excluding false negative results) in the same way as ordinary z-scores. From false*

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			*E. coli* (MF)			Coliform bacteria ("rapid" MPN)			*E. coli* ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2	3	1													0.792	-1.113	-0.407	0.751	0.000	0.000
1237	1	3	2				-0.182	-0.657	-1.769				-0.149	0.000*	0.000	0.296	-0.882		0.277	0.000	0.000
1545	3	2	1				-0.653	-1.250	1.969				-0.600	0.000	1.602	-0.232	-0.328	1.054	-0.228	0.000	0.000
1594	3	2	1				0.044	0.485	0.434				0.067	0.000	-0.202	0.476	-0.221	-0.554	0.450	0.000	0.000
1611	3	2	1				0.393	1.341	0.339				0.401	0.000	0.585	-0.350	0.298	-0.181	-0.341	0.000	0.000
1753	3	2	1				-0.228	0.962	-0.052				-0.193	0.000	0.000	0.296	-0.656	0.973	0.277	0.000	0.000
1868	3	1	2				0.457	1.489	-1.066				0.463	0.000	-1.448	-0.843	0.695	-1.352	-0.813	0.000	0.000
1970	3	1	2				**4.000**	-0.563	-0.052				**4.000**	0.000	-0.523						
2050	2	1	3				-0.414	0.320	0.527				-0.371	0.000	-0.360	-1.397	0.094	0.948	-1.342	0.000	0.000
2386	1	2	3				-1.155	1.039	0.619				0.067	0.000	-0.047	-0.059	1.356	-0.260	-0.062	0.000	0.000
2637	3	1	2													0.653	-0.010	**2.464**	0.277	0.000	0.000
2704	3	1	2				-0.414	-1.902	-0.795				-0.371	0.000	0.683	0.332	1.077	-0.415	0.312	0.000	0.000
2745	2	1	3				0.688	-0.198	0.619				0.683		-0.016						
2944	3	2	1													**-2.684**	-0.031	0.581	**-2.573**	0.000	0.000
3055	3	2	1																		
3076	3	2	1																		
3145	1	3	2													0.186	**2.238**	-1.504	0.172	0.000	0.000
3155	3	2	1				-0.251	-0.198					-0.215	0.000		1.564	-0.010		1.491	0.000	
3159	1	2	3													0.055	0.508	-0.033	0.047	0.000	0.000
3162	1	3	2													0.809	-0.656	-0.782	0.768	0.000	0.000
3164	1	2	3				0.479	1.267	**-4.000**				0.483	0.000	0.000	0.995	-0.115	-1.527	0.946	0.000	0.000
3305	3	1	2				-1.981	-1.677	1.812				-1.870	0.000	0.821	-0.078	-1.719	1.675	-0.080	0.000	0.000
3339	3	2	1																		
3415	2	1	3				0.479	0.236	-0.204				0.483	0.000	0.000	-1.282	1.805	-1.282	-1.233	0.000	0.000
3730	2	1	3																		
3868	2	1	3				-0.900	1.192					-1.870	0.000		0.826	-0.010		0.784	0.000	
3883	3	1	2				**3.521**	**2.260**	1.405				**3.393**	0.000	-0.047	0.111	0.196	1.355	0.101	0.000	0.000
4015	2	3	1													-0.271	0.196	0.973	-0.266	0.000	0.000
4288	1	2	3																		
4319	1	2	3																		
4339	2	1	3				1.482	1.267	-0.256				1.443	0.000	0.000	1.405	-0.882	0.641	**2.757**	0.000	0.000
4343	3	1	2													0.018	0.196	-1.504	0.011	0.000	0.000
4356	1	2	3				1.290	0.403	-0.256				1.259		0.543	-0.078	0.196	**-3.178**	-0.080	0.000	
4633	1	2	3				0.133	0.647	0.527				0.152	0.000	**2.318**	-1.059	-0.545	-0.454	-1.019	0.000	0.000
4723	3	2	1													-1.328	**3.220**	1.351	-1.276	0.000	0.000
4889	1	2	3				1.671	0.151	-0.466				1.623	0.000	0.000	-0.078	0.791		-0.080	0.000	0.000
4980	2	3	1													-1.226	-0.556	**2.443**	-1.179	0.000	0.000
5018	1	2	3				0.791	1.039	-0.466				0.782	0.000	0.000	-0.491	0.196	-0.415	-0.475	0.000	0.000
5120	3	1	2				-1.695	0.566	0.146				-1.596	0.000	0.000	-0.271	-0.768	-0.967	-0.266	0.000	0.000
5128	2	3	1										-1.080	0.000	-0.689	-0.059	0.196	0.808	-0.062	0.000	0.000
5201	2	3	1				-0.068		**-2.098**				-1.814		-0.930						
5220	3	1	2				-0.297	-0.470	**-3.054**				-0.259	0.000	0.000	0.826	-0.882		0.784	0.000	0.000
5352	3	2	1				-1.779	**-2.635**	**-2.070**				**-2.459**	0.000	**-2.179**						
5447	3	1	2				-1.420	**-2.635**	1.570				-1.333	0.000	1.349						
5553	2	1	3					**-4.000**	**-4.000**					0.000	**-4.000**						
5858	3	2	1				0.372	-1.148	1.300				-1.282	0.000	0.000						
5950	1	2	3				0.832	0.065	0.339				0.821	0.000	0.254	1.292	-0.436	**-4.000**	1.230	0.000	0.000
6175	2	1	3													-0.780	-0.545		-0.752	0.000	0.000
6180	1	2	3																		
6182	3	1	2													-1.561	0.196	0.056	-1.499	0.000	0.000
6233	2	1	3				0.264	-0.021	-0.052				0.278	0.000	-0.523	-0.634	-1.350	-1.434	-0.612	0.000	0.000
6253	3	2	1													-0.078	0.094	-0.554	-0.080	0.000	0.000
6448	3	2	1				0.264	0.403	0.339				0.278	0.000	0.000						
6456	1	3	2				-0.653	-0.752	-0.795				-0.600	0.000	0.543	-0.078	-1.113	-0.454	-0.080	0.000	0.000
6563	2	3	1				-0.297	1.267	0.146				-0.259	0.000	0.902	0.441	1.893	0.353	-0.117	0.000	0.000
6686	2	1	3													-0.068	-0.813	-0.415	-0.071	0.000	0.000
7248	3	1	2				0.479	**4.000**	0.291				1.071	0.000	-0.523	1.162	0.597	**-4.000**	1.105	0.000	0.000
7442	3	2	1				0.626	-0.378	0.288				0.624	0.000	0.000	0.223	-1.231	0.109	0.207	0.000	0.000
7688	1	3	2				1.290	0.320	1.152				1.259	0.000	1.476	-0.271	1.264	-0.356	-0.266	0.000	0.000
7728	3	1	2				-1.286	-0.752	-1.769				-1.205	0.000	**-2.223**						
7876	1	2	3				-0.414	0.727	-0.153				-0.371	0.000	-0.047	0.111	0.695	0.210	-0.062	0.000	0.000
7930	1	2	3				0.688	-0.657	-1.141				0.683	0.000	-1.217	0.653	0.196	1.447	0.619	0.000	0.000
7962	1	2	3				-0.414	-0.849	0.978				-0.371	0.000	0.000	1.501	-0.328	0.213	1.430	0.000	0.000
7968	1	3	2				1.093	-0.021	1.321				1.071	0.000	0.105	0.111	-0.997	-0.407	0.101	0.000	0.000
8019	1	3	2				1.482	-0.287	0.048				1.443	0.000	0.000	1.244	-0.221	0.878	1.184	0.000	0.000
8068	1	3	2				-0.825	-0.287	-0.466				-0.764		-0.523	**-3.139**	-1.350	-1.527	**-3.009**	0.000	0.000
8252	2	1	3													1.325	**-2.940**	0.204	1.261	0.000	0.000
8260	1	2	3				0.155	-0.378	-0.287				0.173	0.000	0.060						
8329	2	1	3				0.111	-0.287	0.527				0.131	0.000	-0.860	0.387	1.077	-0.856	0.364	0.000	0.000
8380	2	3	1				**2.038**	1.341	0.339				1.975		1.602	0.826	0.888	0.204	0.784	0.000	0.000
8435	3	1	2				**3.709**	-1.460	-1.024				0.879	0.000	0.000						
8569	2	3	1				1.290	0.403	0.434				1.259	0.000	0.000	0.441	0.196	-1.046	0.416	0.000	0.000
8626	1	2	3				**-2.727**	**-4.000**	0.481				**-4.000**	0.000	-0.124						
8628	3	1	2				0.044	-1.354	-0.574				0.067	0.000	0.000						
8663	3	2	1				**2.038**	1.192	-0.574				1.975	0.000	-0.860	-0.078	-0.436	-0.165	-0.080	0.000	0.000
8742	2	3	1				-0.533	-0.378	-1.024				-0.485	0.000	-1.217						
8751	3	1	2													**-2.347**	1.077	-0.958	**-2.251**	0.000	0.000
8766	1	2	3				0.044	0.065	-0.495				0.067	0.000	1.054	0.111	-0.436	0.425	0.101	0.000	0.000
8840	3	2	1																		
8862	2	3	1				-0.776	-0.021	0.243				-0.717	0.000	0.000	-0.822	1.077	-0.726	-0.792	0.000	0.000
8898	1	2	3				0.264	-0.021	0.261				0.278	0.000	0.051	0.332	0.298	-0.006	0.312	0.000	0.000
8955	2	3	1				-0.900	0.403	1.488				-0.836	0.000	1.349	-0.675	0.398	0.022	-0.652	0.000	0.000
8998	2	1	3																		
9436	3	1	2				0.647	0.236	0.711				0.644	0.000	0.371	0.565	0.298	-0.415	0.535	0.000	0.000
9524	3	1	2				-0.653	-0.563	-0.683				-0.600	0.000	-0.689	0.130	-1.350	-0.655	0.118	0.000	0.000

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

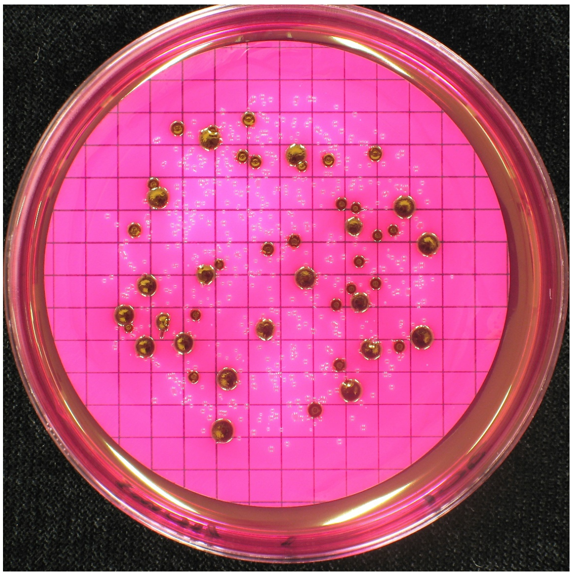
Presumptive *C. perfringens* (MF)			***Clostridium perfringens* (MF)**			**Moulds (MF)**			**Yeasts (MF)**			**Actinomycetes (MF)**			**Total plate count 22 °C, 3 days**			**Lab no.**
A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	
0.000	1.949	-1.173													0.594	-1.379	0.797	**1131**
				0.516	0.000										-0.444	0.480	-0.602	**1237**
0.000	-1.442	-0.758	0.000	-1.279	0.000	0.976	0.000	0.828	-1.371	0.000	0.000	0.000	0.310	0.000	0.869	1.317	0.387	**1545**
						1.478	0.000	0.045	0.173	0.000	0.000	0.000	**-2.466**	0.000	-0.287	0.480	-0.040	**1594**
															0.524	-0.072	0.068	**1611**
0.000	**4.000**	0.130	0.000			-0.146	0.000	-0.794	-0.189	0.000	0.000	0.000	0.052	0.000	0.663	-1.131	0.491	**1753**
0.000	1.734	0.056				-0.737	0.000	**2.548**	-1.941	0.000	0.000	0.000	0.683	0.000	0.310	0.480	0.387	**1868**
0.000	-0.604	-0.157	0.000	-0.423	0.000	-0.300	0.000	0.246	1.959	0.000	0.000				-1.189	**-2.434**	0.282	**1970**
0.000	0.227	0.807				-0.300	0.000	-0.367	0.858	0.000	0.000	0.000	0.182	0.000	-0.134	0.905	1.288	**2050**
0.000	**-2.159**	-0.472	0.000	**-2.012**	0.000										1.459	1.618	-0.040	**2386**
															-1.277	-1.763	-0.150	**2637**
0.000	-0.411		0.000	-0.225	0.000										-1.277	-0.185	-1.076	**2704**
															0.091	0.040	-1.841	**2745**
			0.000	0.246	0.000										-0.210	0.694	-0.836	**2944**
															3.103	-0.072	1.192	**3055**
															-3.102	-1.894	-1.579	**3076**
																		3145
0.000	0.314		0.000	0.516											0.663	0.040		**3155**
			0.000	1.817	0.000										0.732	0.905	-0.602	**3159**
0.000	0.651	-1.040				0.438	0.000	-0.367	-1.162	0.000	0.000	0.000	-0.493	0.000	0.663	-0.072	1.479	**3162**
							0.000		**4.000**			0.000	0.310	0.000	**-2.122**	0.152	-0.373	**3164**
			0.000		0.000	0.296	0.000	-1.238	-0.959	0.000	0.000	0.000	-0.353	0.000	-1.828	0.262	0.068	**3305**
																		3339
0.000	-0.039	1.288	0.000	0.155	0.000	-0.873	0.000	0.637	0.349	0.000	0.000				-0.058	1.009	-0.261	**3415**
															-0.134	-0.415	0.491	**3730**
0.000	-1.009		0.000	-0.837		0.438	0.000		1.185	0.000		0.000	1.155			-1.254		**3868**
0.000	-1.009	1.175				-0.458	0.000	1.742	0.173	0.000	0.000	0.000	-0.353	0.000	-0.210	-0.887	-0.487	**3883**
0.000	1.878	0.603				-0.192	0.000	0.866	1.312	0.000	0.000	0.000	0.182	0.000	-0.134	0.480	1.758	**4015**
																		4288
																		4319
0.000	-0.130	-1.570	0.000	0.061	0.000	0.438	0.000	-1.014	1.185	0.000	0.000	0.000	1.039	0.000	1.201	0.371	0.176	**4339**
0.000	0.815	-1.744				-1.396	0.000	-0.118	**-2.149**	0.000	0.000	0.000	-1.908	0.000	0.091	-0.649	0.387	**4343**
	-1.009		0.000	-0.837	0.000										-1.366	-0.767	0.594	**4356**
						0.239	0.000	0.771	-0.623	0.000	0.000	0.000	**-2.667**	0.000	0.017	-1.008	0.387	**4633**
0.000	0.568	1.036				**2.877**	0.000	-1.943	0.226	0.000	0.000	0.000	-0.353	0.000	-0.766	1.215	1.192	**4723**
			0.000	0.337	0.000										1.711	-0.299	-0.373	**4889**
0.000	-1.788	1.343	0.000	-1.634	0.000										-0.287	0.905	1.384	**4980**
0.000	-0.223	-1.405	0.000	-0.033	0.000	-0.621	0.000	-0.367	-0.006	0.000	0.000	0.000	0.182	0.000	-0.287	-0.185	0.594	**5018**
			0.000	-0.128	0.000	-1.511	0.000	-0.578	0.692	0.000	0.000	0.000	1.039	0.000	-0.210	1.418	-0.150	**5120**
			0.000	-1.054											1.586	0.262	0.491	**5128**
															-1.277		-0.836	**5201**
																		5220
0.000	-0.411	-1.121				1.105	0.000	0.246	-0.565	0.000	0.000	0.000	0.182	0.000	-0.603	0.480	**-2.113**	**5352**
0.000	0.051	0.807	0.000	0.246	0.000	0.576	0.000	-1.238	-0.375	0.000	0.000	0.000	-0.493	0.000	-0.058	0.800	-0.602	**5447**
			0.000	1.817	0.000													**5553**
0.000	**-2.699**		0.000	**-2.564**	0.000										-0.365	-0.887	-0.040	**5858**
0.000	0.400	0.243				0.507	0.000	0.941	-0.281	0.000	0.000	0.000	0.182	0.000	0.732	-0.185	-0.150	**5950**
															0.310	**-4.000**	-0.602	**6175**
																		6180
																	0.068	**6182**
															-1.733	-1.379	**-3.312**	**6233**
						-0.788	0.000	0.246	**4.000**	0.000	0.000				0.453	1.519	0.387	**6253**
															-0.444	-1.131	-0.150	**6448**
															1.201	0.694	0.282	**6456**
0.000	0.485	0.560				-0.891		-0.685	0.384	0.000	0.000	0.000	1.492	0.000	-0.058	0.694	**2.032**	**6563**
0.000	-0.039														0.453	0.587	0.696	**6686**
0.000	0.051	-0.991	0.000	0.246		-1.138	0.000	-1.125	0.692	0.000	0.000	0.000	-0.931	0.000	**-2.223**	**-3.456**	0.491	**7248**
0.000	1.132	-0.906				-1.511		-0.304	0.384	0.000	0.000	0.000		0.000	0.801	0.262	-0.373	**7442**
0.000	0.400	-0.558	0.000	0.603	0.000	0.296	0.000	0.246	-0.760	0.000	0.000	0.000	-0.353	0.000	0.382	-0.415	1.666	**7688**
															0.453	0.800	-1.579	**7728**
0.000	1.210	1.175				0.871	0.000	0.598	-0.623	0.000	0.000	0.000	0.436	0.000	0.382	0.800	0.282	**7876**
0.000	0.400	1.117	0.000	0.603	0.000	0.845	0.000	-0.159	-1.371	0.000	0.000				1.267	0.040	0.282	**7930**
						0.712	0.000	0.045	-1.806	0.000	0.000				0.936	1.618	0.491	**7962**
0.000	0.734	-0.544	0.000	0.944	0.000	-0.458	0.000	0.828	0.173	0.000	0.000				-0.210	0.694	0.696	**7968**
0.000	-0.703	0.494	0.000	-0.524	0.000	0.005	0.000	-0.903							-0.444	-1.634	1.094	**8019**
			0.000	0.860	0.000	0.005	0.000	0.637	-0.565	0.000	0.000				**-2.023**	-1.008	-0.602	**8068**
			0.000	0.944	0.000													**8252**
0.000	-0.411	0.505	0.000	-0.225	0.000										0.382	0.040	-0.040	**8260**
0.000	-0.039	**2.554**				-1.832	0.000	-1.822	-0.189	0.000		0.000	0.683	0.000	0.524	0.371	-0.040	**8329**
															0.869	0.262	-0.955	**8380**
			0.000	-0.323	0.000										0.017	-0.767	-1.841	**8435**
0.000	-0.039	-1.229	0.000	0.155											-0.287	1.913	1.192	**8569**
															-0.932	-1.254	-0.040	**8626**
			0.000	-0.731	0.000	0.712	0.000	-0.514	0.349	0.000	0.000				0.801	0.905	0.068	**8628**
0.000	-0.223	0.494	0.000	-0.033	0.000										0.594	-0.299	**2.732**	**8663**
															1.267	0.371	0.282	**8742**
															-0.603	-0.185	-0.602	**8751**
0.000	0.815	0.039				0.507	0.000	-1.103	0.066	0.000	0.000	0.000	1.381	0.000	0.663	0.262	-0.955	**8766**
																		8840
0.000	1.363	0.173	0.000	1.588	0.000	-0.737	0.000	-0.685	0.226	0.000	0.000	0.000	0.683	0.000	0.017	-0.531	-0.373	**8862**
0.000	-0.604	-0.284				-0.300	0.000	**2.697**	-0.281	0.000	0.000	0.000	0.436	0.000	-0.444	0.262	-0.487	**8898**
			0.000	1.431	0.000	0.712		-0.159	**2.254**	0.000	0.000	0.000	1.381	0.000	-0.210	-0.415	-1.450	**8955**
																		8998
0.000	0.568	0.872				**-2.334**	0.000	0.598	1.136	0.000	0.000	0.000	-0.493	0.000	-0.684	-0.185	-1.199	**9436**
0.000	-0.411	-1.489	0.000	-0.225	0.000										-0.058	1.913	-1.199	**9524**

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**
9736	3	1	2													-0.675	-0.768	0.089	-0.652	0.000	0.000
9899	3	2	1				-1.339	0.566	0.711				-1.256	0.000	-0.360	**2.495**	0.196	**2.136**	**2.381**	0.000	0.000
9903	1	3	2				0.329	0.151	0.275				0.046	0.000	-0.062						
n				0	0	0	60	60	59	0	0	0	61	57	60	64	64	58	64	64	61
Min							-2.727	-4.000	-4.000				-4.000	0.000	-4.000	-3.139	-2.940	-4.000	-3.009	0.000	0.000
Max							4.000	4.000	1.969				4.000	0.000	2.318	2.495	3.220	2.464	2.757	0.000	0.000
Median							0.122	0.022	0.146				0.067	0.000	0.000	0.083	0.042	-0.220	-0.026	0.000	0.000
Mean							0.187	-0.067	-0.136				0.056	0.000	-0.067	0.000	0.000	-0.193	0.000	0.000	0.000
SD							1.276	1.326	1.224				1.293	0.000	0.972	1.000	1.000	1.284	1.000	0.000	0.000
z<-3							0	2	3				1	0	1	1	0	3	1	0	0
-3≤z<-2							1	2	2				1	0	2	2	1	0	2	0	0
2<z≤3							2	1	0				0	0	1	1	1	3	2	0	0
z>3							3	1	0				2	0	0	0	1	0	0	0	0

Presumptive *C. perfringens* (MF)			*Clostridium perfringens* (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	
0.000	0.227					1.478	0.000	0.771	1.152	0.000	0.000	0.000	-1.084		0.732	-0.415	-0.373	**9736**
0.000	-1.221	-0.253				0.576	0.000	0.045	-0.319	0.000	0.000	0.000	-0.353	0.000	-0.058	0.371	0.594	**9899**
0.000	0.651	0.223				0.438	0.000	-0.097	0.607	0.000	0.000	0.000	0.310	0.000	0.524	-0.072	1.094	**9903**
44	45	38	36	35	31	42	40	41	42	41	39	34	33	32	76	76	76	**n**
0.000	-2.699	-1.744	0.000	-2.564	0.000	-2.334	0.000	-1.943	-2.149	0.000	0.000	0.000	-2.667	0.000	-3.102	-4.000	-3.312	**Min**
0.000	4.000	2.554	0.000	1.817	0.000	2.877	0.000	2.697	4.000	0.000	0.000	0.000	1.492	0.000	3.103	1.913	2.732	**Max**
0.000	0.051	0.093	0.000	0.061	0.000	0.122	0.000	-0.097	0.173	0.000	0.000	0.000	0.182	0.000	-0.021	0.040	0.014	**Median**
0.000	0.089	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.000	0.000	0.000	-0.053	0.000	**Mean**
0.000	1.154	1.000	0.000	1.000	0.000	1.000	0.000	1.000	1.302	0.000	0.000	0.000	1.000	0.000	1.000	1.094	1.000	**SD**
																		Sum
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	16
0	2	0	0	2	0	1	0	0	1	0	0	0	2	0	3	1	1	26
0	0	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	2	18
0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	11

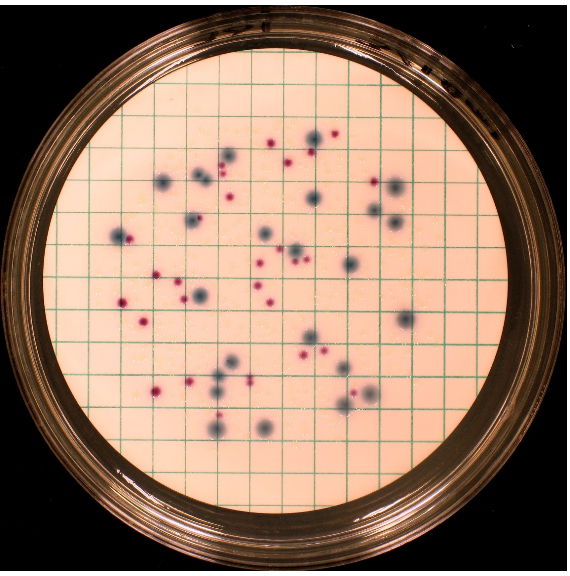
Mixture A

m-Endo Agar LES, 37 °C



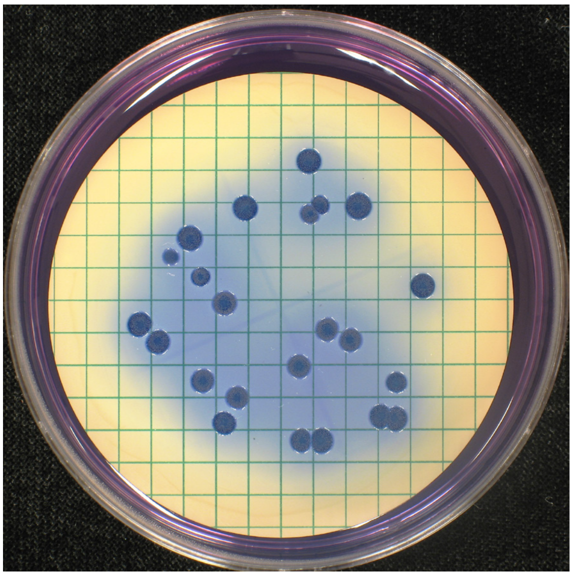
10 ml

Chromocult Coliform Agar, 37 °C



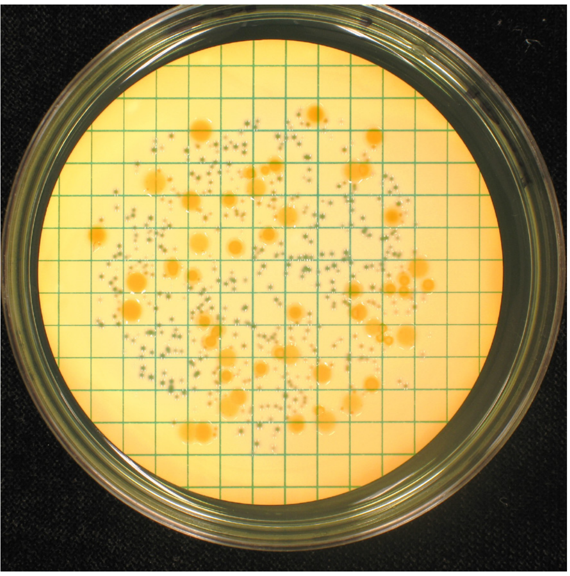
10 ml

m-FC Agar, 44 °C



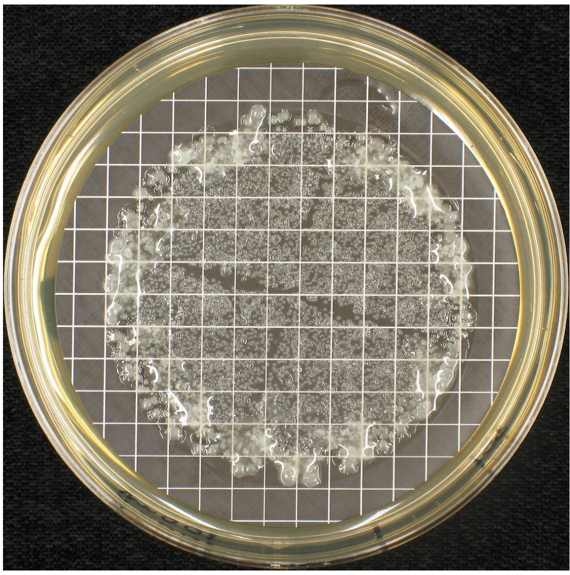
10 ml

m-Lactose TTC Agar, 37 °C



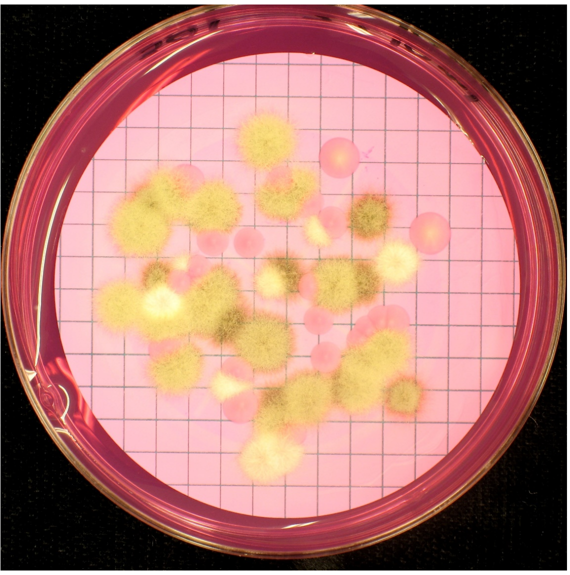
10 ml

Actinomycete Isolation Agar, 25 °C



100 ml, 7 days

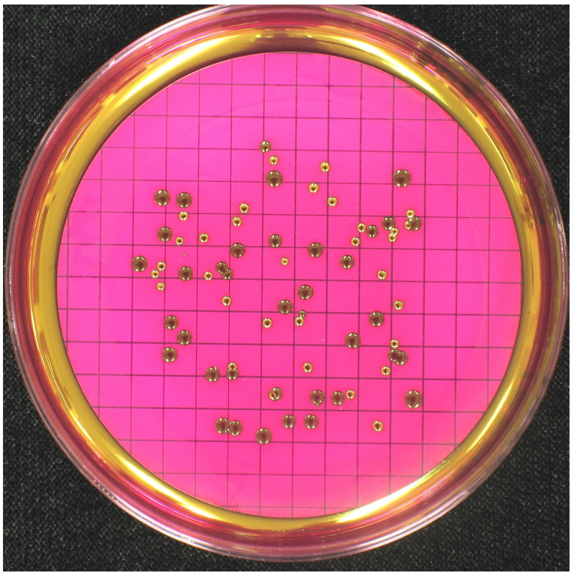
m-RBCC Agar, 25 °C



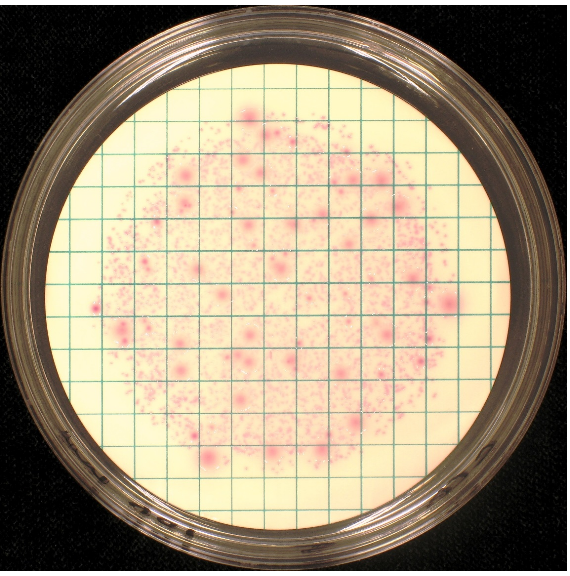
10ml, 7 days

Mixture B

m-Endo Agar LES, 37 °C



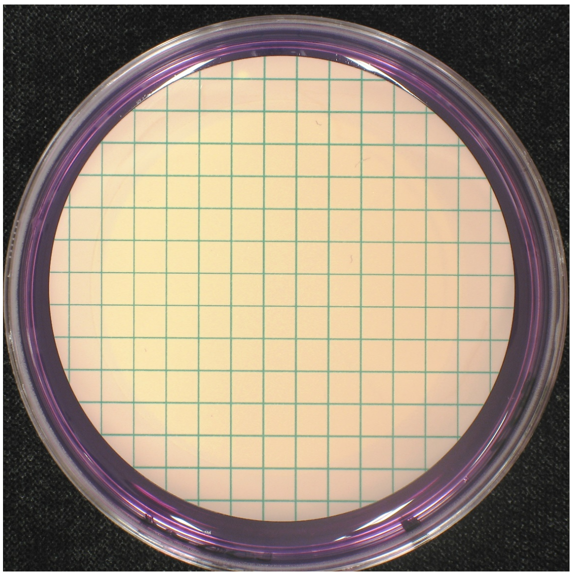
100 ml



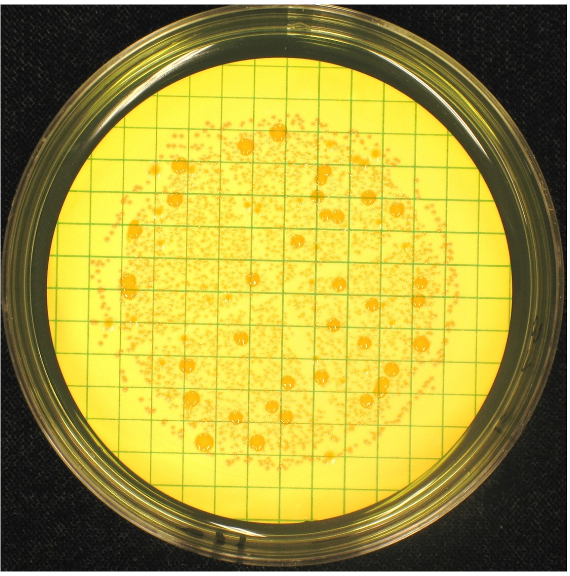
100 ml

Chromocult Coliform Agar, 37 °C

m-FC Agar, 44 °C



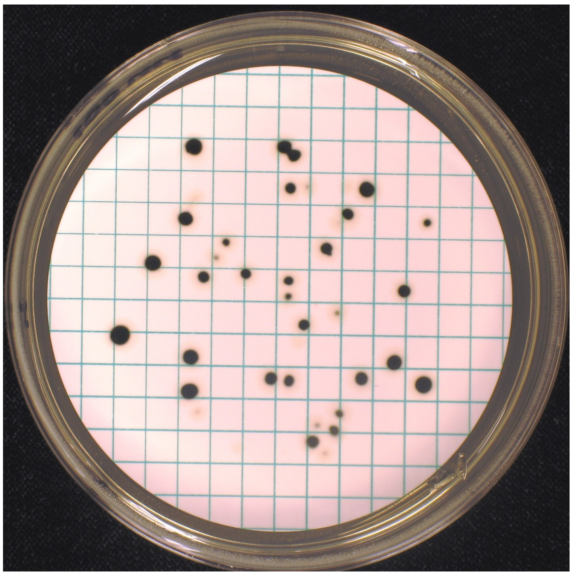
100 ml



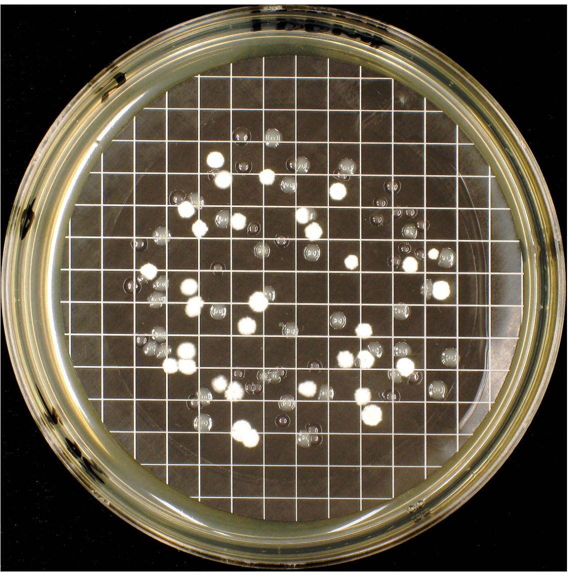
100 ml

m-Lactose TTC Agar, 37 °C

m-TSC Agar, 44 °C



100 ml

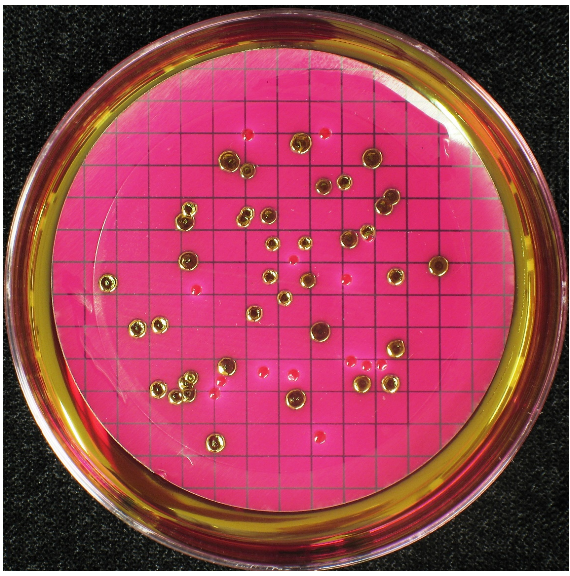


100 ml, 7 days

Actinomycete Isolation Agar, 25 °C

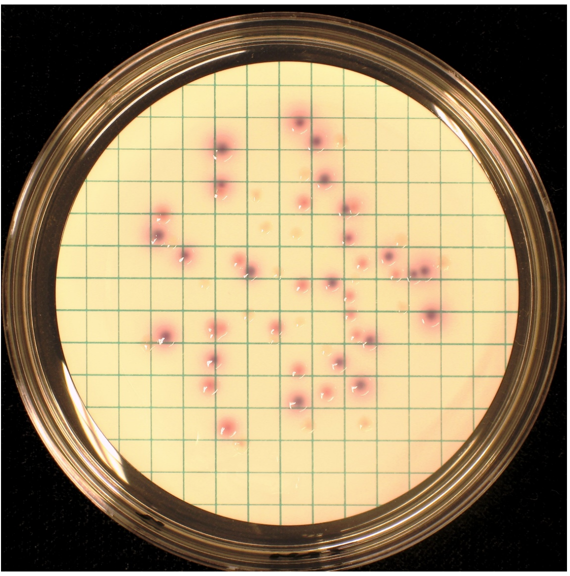
Mixture C

m-Endo Agar LES, 37 °C



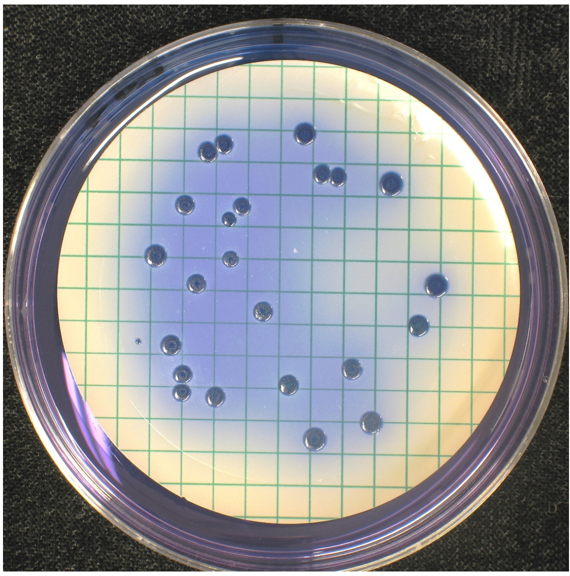
1 ml

Chromocult Coliform Agar, 37 °C



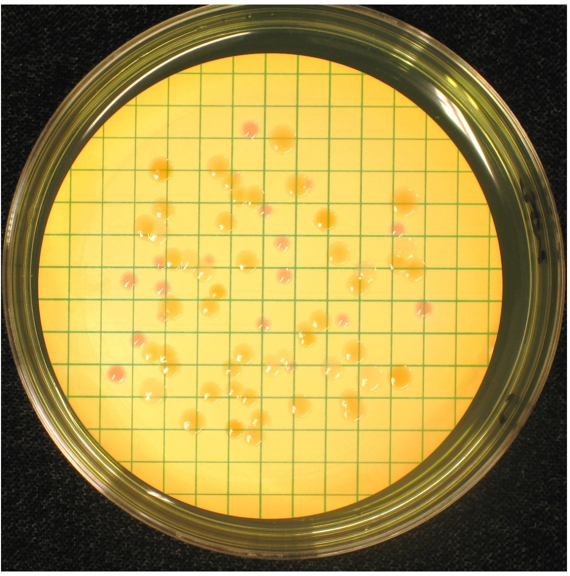
1 ml

m-FC Agar, 44 °C



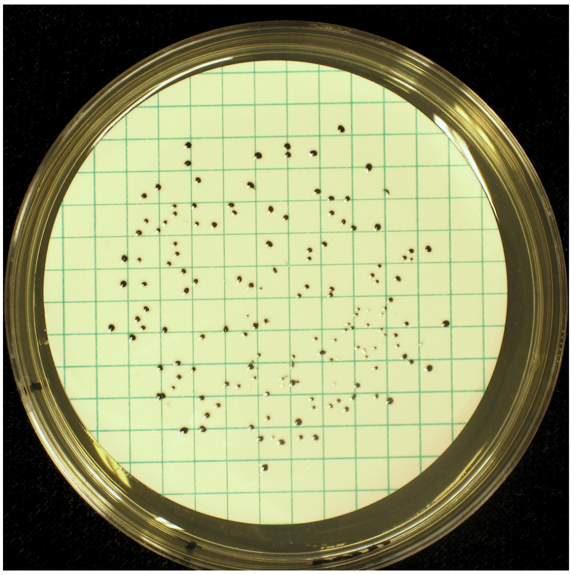
1 ml

m-Lactose TTC Agar, 37 °C



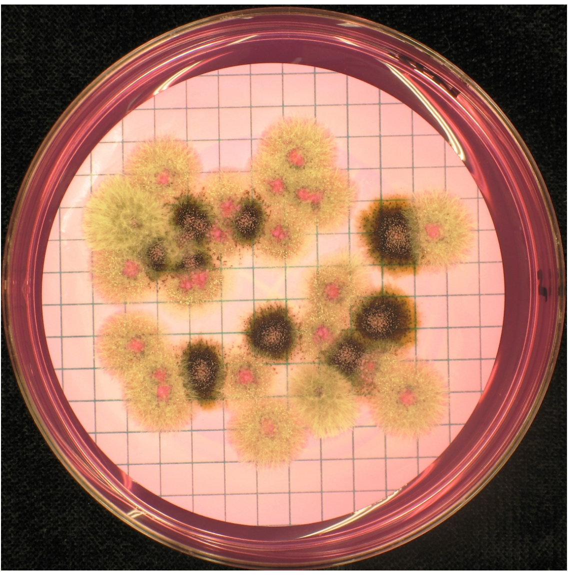
1 ml

m-TSC Agar, 44 °C



10 ml

m-RBCC Agar, 25 °C



10 ml, 7 days

PT reports published 2017

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

Proficiency Testing – Drinking Water Microbiology, March 2017, by Tommy Šlapokas

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

Proficiency Testing – Drinking Water Microbiology, September 2017, by Tommy Šlapokas

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

PT reports published 2018

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

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

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

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

The National Food Agency's PT program offers

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

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

The National Food Agency's reference material

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

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