Drinking Water Microbiology March 2018

Tommy Šlapokas







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



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

Contents

Abbreviations and explanations	2
Contents	3
General information on results evaluation	4
Results of the PT round	4
- General outcome	4
- Coliform bacteria (MF)	6
- Suspected thermotolerant coliform bacteria (MF)	8
- Escherichia coli (MF)	
- Coliform bacteria and E. coli (rapid method, MPN)	12
- Presumptive and confirmed Clostridium perfringens (MF)	15
- Moulds and yeasts (MF)	17
- Actinomycetes (MF)	
- Culturable microorganisms 22 °C, 3 days	21
Outcome of the results and laboratory assessment	
- General information about reported results	
- Base for assessment of the performance	
- Mixed up results and other practical errors	
- Z-scores, box plots and deviating results for each laboratory	
Test material, quality control and processing of data	
- Description of the test material	
- Quality control of the test material	
- Processing of numerical results	30
References	31
Annex A – All reported results	32
Annex B – Z-scores of the results	
Annex C – Photo example of colony appearance on some media	40

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 (<u>www2.slv.se/absint</u>).

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

Mixture В С А ^{5%} 0% 4% 2% 7% 2% 0% Percentage of laboratories with 15% 0 deviating results 1 deviating result 83% 88% 87 2 deviating results >2 deviating results 529 525 507 No. of evaluable results No. of deviating results 14 (3 %) 15 (3 %) 18 (4 %) Escherichia coli Citrobacter freundii Microorganisms Escherichia coli Enterobacter cloacae Aeromonas hydrophila Klebsiella oxytoca Candida glabrata Clostridium perfringens Hafnia alvei Phialophora fastigiata Streptomyces sp. Clostridium bifermentans **Stenotrophomonas** Staphylococcus Phoma glomerata maltophilia saprophyticus Analysis F% X% Target org. F% X% F% X% Target org. Target org. E. coli 2 5 5 E. coli 3 Coliform bacteria 0 0 C freundii (MF) [A. hydrophila] K. oxytoca E. cloacae Susp. thermotolerant E. coli E. coli _ _ _ _ coliform bact. (MF) {E. cloacae} 7 E. coli 2 5 E. coli 2 E. coli (MF) _ 30 [E. cloacae] C freundii 5 Coliform bacteria E. coli 0 0 0 0 0 E. coli K. oxytoca (rapid method) E. cloacae E. coli (rapid meth.) E. coli 0 0 0 0 E. coli 2 0 Presumptive C. 0 2 С. 10 2 _ C. perfringens 0 perfringens (MF) bifermentans Clostridium 3 C. perfringens 3 0 [C. biferment.] 9 _ _ _ perfringens (MF) Actinomycetes (MF) 25 °C 0 _ Streptomyces sp. 0 0 3 _ 25 °C Ph. fastigiata 2 7 _ Ph. glomerata 2 Moulds (MF) 0 0 25 °C C. glabrata 5 2 Yeasts (MF) 0 5 _ Culturable micro-22 °C S. maltophilia 1 0 S. saprophyticus 0 1 H. alvei 0 0 (C. freundii) E. coli organisms (total E. coli (K. oxytoca) E. cloacae count), 3 days A. hydrophila

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*

* 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.

Madium	N			Α						В						С			
wiedium	IN	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





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	Α			В		С
	n	n Med C	V F < >	n Med	CV F < >	n Me	d CV F $< >$
Total	27	27 150		27 0		- 25 230	0

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



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

Onigin & Standard	N			Α					В					С			
Origin & Standard	1	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
<u>Colony origin</u>																	
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 (8	0	_	1		5	2445	11	3	0	0
Other/Unknown	0	0	-	_	_		- 0	_	_	_		0	-	_	_	_	_
<u>Standard</u>																	
ISO 9308-1:2000	0	0	_	_	_		- 0	_	_	_		0	_	_	_	_	_
ISO 9308-1:2014	26	25	180	13	1	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 (13	0	_	3		11	2720	12	4	0	0
NS 4792	2	2	-	_	0	0 (2	0	_	0		2	-	_	0	0	0
Other/Unknown	3	1	-	_	0	1 1	3	0	_	0		2	-	_	1	0	0

All results

Madium	N			Α						В					С			
Medium	IN	n	Mv	CV	F	<	\vee	n	Mv	CV	F	< >	n	Mv	CV	F	<	>
Total	<i>47</i> [#]	45	<i>189</i>	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

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

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.

In and attack times	NI			Α						В						С			
incudation time	IN	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	$^{\prime}$	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

Coliform bacteria, Rapid method with MPN

Е.	coli.	Ravid	method	with	MPN
	•••••	1.0000000			

Insubstion time	N			Α						В					С			
incubation time	19	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	_	Ι
(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



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*.

Standard/Mathad	N#			Α						В						С			
Standard/Method	IN	n	Mv	CV	F	<	: >	n	Mv	CV	F	<	$^{\prime}$	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	-	-	_	_	_

Presumptive Clostridium perfringens MF

Clostridium perfringens MF

Standard/Mathad	N#			Α					В					С			
Standard/Iviethod	IN	n	Mv	CV	F	< >	n	Mv	CV	F	< >	· n	Mv	CV	F	<	>
Total	58	36	0	-	1		35	38	14	1	0 (31	0	-	3	-	-
(EN) ISO 14189	44	25	0	_	1		24	39	13	1	0 (22	0	-	2	-	-
ISO/CD 6461-2:2002	10	7	0	_	0		7	40	14	0	0 (6	0	_	1	_	_
m-CP agar, EU-direct.	4	4	0	_	0		4	30 [*]	_	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.

Standard/Mathad	N			Α						В						С			
Standard/Iviethod	IN	n	Mv	CV	F	< 2	>	n	Mv	CV	F	<	$^{\prime}$	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	_	Ι	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

Moulds MF

Yeasts MF

Stor doud/Mothod	N			Α						В						С			
Standard/Method	IN	n	Mv	CV	F	<	$^{\vee}$	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.

Madium/Standard	N			Α					В						С		
Medium/Standard	IN	n	Mv	CV	F	< >	n	Mv	CV	F	<	$^{\prime}$	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.

Caroan of an aulta	NI			Α						В						С			
Group of results	IN	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	$^{\prime}$	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
<u>Medium</u>																			
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	4 7 [*]	-	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.



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











Test material, quality controls and processing of data

Description of the test material

This round comprised three test items with different microorganism 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.

Mixture ¹	Microorganisms	Strain co	llection no.	cfu/100 ml ²
		SLV (own)	Reference ³	
А	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 *
В	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*
С	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

Table 2 Microorganisms present in the mixtures

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%).

Analysis parameter				Mi	xtur	e			
Method standard for analysis		\mathbf{A}^1			\mathbf{B}^{1}			\mathbf{C}^2	
	cfu	I ₂	Т	cfu	I ₂	Т	cfu	I ₂	Т
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	22 ^a	0.6	1.4	63	0.3	1.2	39 ^b	1.9	1.6
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	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

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

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 <u>www2.slv.se/absint</u>.

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, <u>http://www.standardmethods.org/</u>
- 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 <math>< '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	Suspec	ted col	iform F)	Coliform	h bacter	ia (MF)	Susp. th	nermoto m bact.	lerant (MF)	Ε.	coli (Mi	F)	Colifo	orm bac pid" Mi	teria N)	E. coli	("rapid"	MPN)
	ABC	A	B	<u>с</u>	A	в	С	A	B	C	A	в	С	A	B	<u>с</u>	Α	в	С
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	321	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	321	500	62	3900	206	62	3900	150	0	2700	206	0	2730	186	51	3683	186	0	0
1753	321	361	57	3500	178	57	3500	-	-	-	178	0	4477	220	42	5000	220	0	0
1868	312	209	64 20	2564	209	64 20	2564	- E20	-	2500	209	0	1477	162	55	2551	162	0	0
2050	212	520	- 39	3300	170	39 40	3300 4100	520	0	3300	170	0	2000	137	- 40	4970	137	-	-
2386	$\frac{2}{123}$	250	58	4200	140	49 58	4200	190	0	2300	190	0	2300	201	49 62	3600	201	<1	<1
2637	3 1 2	- 200	-		-	-	.200	-	-	- 2000	-	-	- 2000	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	321	-	-	-	-	-	-	-	-	-	-	-	-	87	47.8	4530	87	<1	<1
3055	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	132	-	-	-	-	-	-	-	-	-	-	-	-	214	72	2420	214	0	0
3155	321	332	43	-	177	43	-	140	<1	-	177	<1	-	295	48 52 1	-	295	<1	- 1
3159	123	-	-	-	-	-	-	-	-	-	-	-	-	207	33.1	3040	207	<1	<1
3164	123	-	-	-	210	61	240	-		-	210	0	0	249	42	2400	249	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	231	-	-	-	-	-	-	-	-	-	-	-	-	190	50	5000	190	0	0
4288	123	24	52	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	123	- E 4 0	- 61	-	-	- 61	-	-	-	-	-	-	-	-	-	4600	205	-	-
4339 4343	213	540		3300	200		3300	140	0	3200	200	0	0	205	40 50	2/20	205	0	0
4356	123	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	Ő	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	123	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	231	-	-	-	105	-	1760	-	-	-	140	<1	1900	201	50	4800	201	<1	<1
5201	2 3 1 2	-	-	-	105	40	1150	-	-	-	175	-	1760	250	40	> 2410	250	-	-
5352	321	-	-	-	117	20	1780	-	-	-	90	<1	1120	- 200		- 110	- 200	-	-
5447	3 1 2	-	-	-	130	20	5300	-	-	-	130	0	3300	-	-	-	-	-	-
5553	2 1 3	-	-	-	0	10	380	-	-	-	0	0	240	-	-	-	-	-	-
5858	321	-	-	-	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	123	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6182	312	-	-	-	-	-	-	-	-	-	-	-	-	130	50	3937	130	0	0
0233 6253	2 1 3	300	45	3500	200	45	3500	-	-	-	200	0	2000	200	30	2480	200	0	0
6448	321	4200	50	3900	200	50	3900	-		-	200	0	0	200	49		200	-	-
6456	1 3 2	-200	-	- 3300	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	321	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	312	-	-	-	135	37	2000	-	-	-	135	0	1100	-	-	-	-	-	-
7876	123	409	54	3400	170	54	3400	109	<1	1864	170	<1	2300	210	55	4106	201	<1	<1
7930	123	220	30	2500	220	30	2500	124	-	2400	220	0	1600	240	20	3600	240	0	0
7968	1 2 3 1 3 2	490	45	5000	240	45	5000	124	0	2400	240	0	2400	231	39	3448	291	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.

Presi	umptive	C.	Clo	stridiu	m	Moulds (MF)			Ye	asts (MI	=)	Actino	mycetes	(MF)	Total	plate co	ount	Lab no.
perfri	ngens (MF)	perfri	ngens	(MF)	•	в	~	•	в	~	•	в	~	22±2	°C, 3 da	ys#	
A 0	65	240	A .	<u>в</u> -		A -	<u>в</u> -	<u> </u>	A .	<u>в</u> -		A -	<u>ь</u> -	<u> </u>	A 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
-	400	-	-	-	-	-	-	-	-	-	-	-	-	-	63	56	46	1611
0	120	2200	0	-	-	210	0	250	250	0	0	0	29	0	60 60	47	50 40	1/53
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	30	<1	<1	36	<1	-	-		-	-	-	-	-	-	40	55 57	30	2704
-	-	-	<1	41	<1	-	-	-	-	-	-	-	-	-	53	63	38	2944
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	105	56	57	3055
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	41	32	3076
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3145
<1	44	-	<1	44 60	- 1	-	-	-	-	-	-	-	-	-	65	57 65	- 40	3155
0	48	350	-	- 00	-	250	0	270	200	0	0	0	25	0	65	56	40 60	3162
-	-	-	-	-	-	0	Ő	0	600	65	450	0	31	Ő	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 52	43	3415
- 0	30	-	- 0	30	-	- 250	-		- 330	-	-	- 0	- 38	-	54	53 46	50	3730
0	30	5200	-	-	-	190	0	380	270	Ő	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 50	30	-	- 39	-	250	0	240	330	0	0	0	37	0	73 57	60 51	47	4339 4343
3100	30	Ő	0	30	0	-	-	- 202	-	-	-	-	-	-	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	190	-	- 270	-	-	-	-	- 20	-	52 52	65 55	59 51	4980
-		- 30	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 64	28	5352
-		4000	0	60	0	200	-	230	240	-	-	-	- 25	-		- 04	40	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 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 62	66 52	6563
0	40	395	0	41	395	150	- 0	235	300	0	-	0	- 22	- 0	30	30	50	7248
0	54	481	-		-	130	1	273	282	Ő	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 57	48 40	7876
-	40 -	5000	-	40 -	0	260	0	200 20∩	190	0	0		-	-	74 69	51 72	40 50	7950
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	-	-	<1	49	<1	-	-	-	-	-	-	-	-	-	- 61	-	-	8252
<1 0	30 40	1916	< 0	38	< 0	220	-	288	260	-	-	0	- 29	0	56	57	40 45	0200 Mean
-	14	55	-	14	-	15	-		11	-	-	-	13	-	12	8	10	CV (%)

Lab no.	Sample	Suspec	ted col	iform	Coliforn	n bacter	ia (MF)	Susp. th	ermoto	olerant	E.	coli (M	F)	Colif	orm bac	teria	E. coli	("rapid"	MPN)
		bac	teria (M	F)		-		CONTOR	m bact.	(MF)		-	~	("ra		PN)		-	
0000	ABC	A	В 40	U 44.00	A	В 40	1100	~	В	U	A	Б	1000	A	Б	0005	A	Б	U
8329	213	193	42	4100	193	42	3000	-	-	-	193	24	1800	225	59	3005	225	0	0
0300 9435	2 3 1 2	510	02	3900	290	20	2600	100	-	2000	290	24	3500	250	57	4100	250	0	0
8569	2 3 1	250	50	4000	250	50	2000	105		2000	250	0	0	228	50	2827	228	-	-
8626	123	430	46	4500	86	9	4050	43	0	2250	43	0	2250	- 220		2027	- 220	-	-
8628	3 1 2				190	31	3000	110	0	1600	190	Ő	0	-	-		-	-	-
8663	321	490	60	3000	290	60	3000	150	Ő	2400	290	0	1800	200	44	3700	200	0	0
8742	2 3 1	-	-	-	165	41	2600	-	-	2.00	165	Ő	1600	- 200	-	-	- 200	-	-
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	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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	312	382	48	4300	218	48	4300	209	0	3300	218	0	2580	235	51	3440	235	0	0
9524	312	310	39	2900	160	39	2900	-	-	-	160	<1	1900	211	36	3200	211	<1	<1
9736	312	-		-	-	-	-	-	-	-	-	-		170	41	3973	170	0	0
9899	321	133	52	4300	133	52	4300	-	-	-	133	0	2100	357	50	6532	357	0	0
9903	132	411	47	3833	203	47	3833	189	0	3370	189	0	2290	-	-	-	-	-	-
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		20	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	Ő	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	-	-
L .											-		-	-			-	-	
False po	ositive				0	0	0				0	4	0	0	0	0	0	0	1
False ne	gative				1	0	0				1	0	18	0	0	0	0	0	0
Outliers	, IOW bigh				0	2	2				1	0	1	0	0	3	0	0	0
Outliers	, nign				3	1	0				2	0	0	0	0	0	0	0	0
Low lim		24	32	22	96	20	1150	12	0	0	00	0	1100	72	24	2400	72	0	0
High lim	it OK	4200	531	5800	290	75	5800	520	0	4600	290	0	4100	357	84	7000	385	0	0
									-									-	-
mv					13.712	6.727	59.598				13.670	0.000	48.274	14.286	6.935	62.258	14.297	0.000	0.000
(√Mean)																		
s (C\/*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 rat my (~) %)				1.6	1.7	1.9				1.6		2.2	1.6	1.2	1.9	1.7		
(100*s/ 1	/n _{mv})																		
X (1/Result	(+)																		
z rresul	y																		
([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. Clostridium					Мс	Julds (M	F)	Ye	asts (M	.F)	Actino	myceter	s (MF)	Total	plate cr	ount	Lab no.	
perfr	ingens	(MF)	perfr	ingens /	(MF)	1		· 1	1		<i>'</i> ।	1	•	``I	22+2	.°C,3 d≆	avs [#]	1
Α		<u> </u>	Α	В	С	Α	В	С	Α	В	С	Α	В	С	<u>A</u>	B	<u>C</u>	I'
0	40	11100	-	-		114	0	205	250	0/	132	0	34	0	63	60	45	8329
- 1	-	- '		-	- 1	1 -	-	- 1	1 -	-	- J	1 -	-	- 1	68	59	37	8380
-	-	- '	0	35	0	1 -	-	- 1	1 -	-	- 1	1 -	-	- 1	56	50	30	8435
0	40	200	0	40	200		-	- 1	1 -	-	- 1	1 -	-	- 1	52	75	57	8569
- 1	-	- '	1 :	-	- 1	1	-	-	1	-	- 1	1 -	-	- 1	44	46	45	8626
1 :	-		0	31	01	270	0	263	280	0	01	1 -	-	- 1	67	65	46	8628
0	38	3100	0	38	01	1 -	-	- 1	1 -	-	- 1	1 -	-	- 1	64	54	74	8663
- 1	-	- 1	1 -	-	- 1	1 -	-	- 1	1 -	-	- 1	1 -	-	- 1	74	60	48	8/42
-	-		1 -	-	- 1	255	-		1 264	-	- 1	1	-	-1	48	55	40	8/51
U	50	2000	1	-	- 1	255	U	230	264	U	υj	U U	40	U I	60	59	31	8840
	-	2300		-	- 1	172	-	255	1 272	-	- 1	1	- 24	-1	56	- 52	42	8967
0	57 34	1364		57	01	200	0	200	2/3	0	0		34	ů,	50	52 50	4∠ i //1	8895
	34	1304		- 55	- 1	200	280	280	400	0	0	1	3∠ 40	ů,	53	53 53	41	8055
1 [-			- 55	-	2/0	200	200	400	-	· ·		40	<u> </u>	- 55	- 55	ر دن -	8998
0	47	4200	1 _	-	!	91	0	318	327	0	0	0	25	0	47	55	35	9436
<1	36	62	<1	36	<1	Ŭ .	-	<u> </u>	I -	-	۲	1 Ĭ	-	۲	55	75	35	9524
0	43	0	1	-	<u> </u>	330	0	327	328	0	0	0	21	1182	66	53	42	9736
Ō	28	1419	1 -	-	- '	260	0	290	243	0	ō	l o	26	0	55	60	51	9899
0	48	2417	1 -	-		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
1			1		I	1		1	1		I	1		1	1		1	1
0	40.5	2118	0	39		228	0	283	267	0		0	30	0	55.5	57	45.5	Median
U	40	1916	U	38	U	220	U	288	260	U	U	U	29	U	56	5/	45	Mean
	14	55		14		15		Э	11			i	13	ı	12	8	10	CV (%)
1	0	0	1	0	2	1	2	ر ۱	1	1	ا _د ا	1	0	1	1	0	0	False nos
	0	4		1	31		0	1	1 0	1	<u> </u>		1	<u>ا</u> `		0	0	Faise pos.
Ň	0	÷.		0	0	1 .	0	, i	1 0	0	0	1	0	ů,	1 .	1	0	Faise neg.
ŏ	1	0	l õ	0	ŏ	I õ	ő	ŏ	1 2	ő	ŏ	I õ	ő	õ	ιŏ	0	0	Outliers >
Ĭ	•		Ĩ	•	۲	1 Ť	-	۲	1 -		۲	1 Ť	-	٦	Ĭ	-	~	Guine. C .
0	16	3	0	16	0	91	0	200	155	0	0	0	12	0	22	30	20	Low limit
ŏ	65	11100	Ŏ	60	ŏ	455	õ	436	400	õ	ŏ	l õ	41	ŏ	105	75	74	High limit
			<u> </u>		<u> </u>						<u> </u>		<u> </u>	<u> </u>			<u> </u>	
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
l			1		!	1		!	1		!	1		'	I			1
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	t	2.3		2.4		1.3	1.7			<u> </u>	2.3	\rightarrow	1.4	0.9	1.2	u _{rel.mv} (%)
Ĺ						1			1			1			I			
Ē	_		Ī.	_		Ē	_	Ī	1	_		Ē	_	_ I	1	_		×
					+				[+			+	[z
1			1			1						1			1			1

Lab no.	Sample	Suspected coliform	Colif	orm bac	teria	Susp. thermotolerant	E.	coli (M	F)	Colif	orm bac	teria	E. coli	("rapid'	' MPN)
	ABC	bacteria (MF)	Δ	(MF) B	C	Coliform bact. (MF)	Δ	в	0	("ra ∆	apid" Mi B	² N) C	Δ	в	C
1131	231		~	-	<u> </u>	<u> </u>	~	-	<u> </u>	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 1594	321		-0.653	-1.250	1.969		-0.600	0.000	1.602	-0.232	-0.328	1.054	-0.228	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	321		-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 1970	312		0.457	1.489	-1.066		0.463	0.000	-1.448	-0.843	0.695	-1.352	-0.813	0.000	0.000
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	123		-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 2704	312 312		-0.414	-1.902	-0.795		-0.371	0.000	0.683	0.653	-0.010	-0.415	0.277	0.000	0.000
2745	2 1 3		0.688	-0.198	0.619		0.683		-0.016						
2944	321									-2.684	-0.031	0.581	-2.573	0.000	0.000
3055	321														
3145	1 3 2									0.186	2.238	-1.504	0.172	0.000	0.000
3155	321		-0.251	-0.198			-0.215	0.000		1.564	-0.010	0 022	1.491	0.000	0.000
3162	1 3 2									0.000	-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	312		-1.981	-1.677	1.812		-1.870	0.000	0.821	-0.078	-1.719	1.675	-0.080	0.000	0.000
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		0.000	4 4 9 5			1.070	0.000		0.000	0.015		0.70/	0.000	
3868	213 312		-0.900	1.192	1 405		-1.870	0.000	-0 047	0.826	-0.010	1 355	0.784	0.000	0 000
4015	231		0.021				0.000	0.000	0.071	-0.271	0.196	0.973	-0.266	0.000	0.000
4288	1 2 3														
4319 4339	1 2 3 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			1.201	0.200			0.000	0.000	0.018	0.196	-1.504	0.011	0.000	0.000
4356	1 2 3		1.290	0.403	-0.256		1.259	0.000	0.543	-0.078	0.196	-3.178	-0.080	0.000	0.000
4633 4723	1 2 3 3 2 1		0.133	0.647	0.527		0.152	0.000	2.318	-1.328	-0.545 3.220	-0.454	-1.019	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	231		0 701	1 020	0.466		0 792	0.000	0.000	-1.226	-0.556	2.443	-1.179	0.000	0.000
5018	3 1 2		-1.695	0.566	0.1466		-1.596	0.000	0.000	-0.491	-0.768	-0.415	-0.475	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 5220	231		-0.068	-0.470	-2.098		-1.814	0 000	-0.930	0.826	0 883		0.784	0 000	0.000
5352	3 2 1		-1.779	-2.635	-2.070		-0.259 -2.459	0.000	-2.179	0.020	-0.002		0.764	0.000	0.000
5447	3 1 2		-1.420	-2.635	1.570		-1.333	0.000	1.349						
5553 5858	213		0 372	-4.000	-4.000		-1 282	0.000	-4.000						
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
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 6448	321		0.264	0 /03	0 330		0.278	0 000	0 000	-0.078	0.094	-0.554	-0.080	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	231		-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 7248	213		0 479	4 000	0 291		1 071	0 000	-0 523	-0.068	-0.813	-0.415	-0.071	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	132		1.290	0.320	1.152		1.259	0.000	1.476	-0.271	1.264	-0.356	-0.266	0.000	0.000
7876	123		-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	123		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 7968	123		-0.414	-0.849	0.978		-0.371	0.000	0.000	1.501	-0.328	0.213	1.430	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 8260	213 123		0.155	-0.378	-0.287		0.173	0.000	0.060	1.325	-2.940	0.204	1.261	0.000	0.000
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	231		2.038	1.341	0.339		1.975	0.000	1.602	0.826	0.888	0.204	0.784	0.000	0.000
8435 8569	231		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 8662	312		0.044	-1.354	-0.574		0.067	0.000	0.000	-0 079	-0 136	-0 165	-0 090	0 000	0 000
8742	231		-0.533	-0.378	-1.024		-0.485	0.000	-1.217	-0.070	-0.430	-0.100	-0.000	0.000	0.000
8751	3 1 2									-2.347	1.077	-0.958	-2.251	0.000	0.000
8766 8840	123		0.044	0.065	-0.495		0.067	0.000	1.054	0.111	-0.436	0.425	0.101	0.000	0.000
8862	231		-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 8998	231 213		-0.900	0.403	1.488		-0.836	0.000	1.349	-0.675	0.398	0.022	-0.652	0.000	0.000
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

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

positive results no z-scores can be calculated. Z-scores form outliers are not real zscores 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.

Pres	sumptive ringens	eC. (MF)	Cl	ostridiu inaens	m (MF)	Mo	oulds (N	AF)	Ye	asts (M	F)	Actino	omycete	s (MF)	Total	l plate c °C. 3 da	ount	Lab no.
A	B	<u>с</u>	A	B	<u>c</u>	Α	в	С	Α	В	С	Α	В	С	Α	B	<u>с</u>	
0.000	1.949	-1.173													0.594	-1.379	0.797	1131
0.000	-1 //2	-0 758	0.000	0.516	0.000	0.976	0 000	0.828	-1 371	0 000	0.000	0.000	0 310	0 000	-0.444	0.480	-0.602	1237
0.000	-1.442	-0.750	0.000	-1.275	0.000	1.478	0.000	0.020	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	-0.604	-0.157	0.000	-0.423	0.000	-0.300	0.000	0.246	1.959	0.000	0.000	0.000	0.005	0.000	-1.189	-2.434	0.387	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
0.000	-0.411		0.000	-0.225	0.000										-1.277	-0.185	-1.076	2037
															0.091	0.040	-1.841	2745
			0.000	0.246	0.000										-0.210	0.694	-0.836	2944
															-3.102	-1.894	-1.579	3076
0.000	0.044		0.000	0.540											0.000	0.040		3145
0.000	0.314		0.000	0.516	0 000										0.663	0.040	-0.602	3155
0.000	0.651	-1.040	0.000	1.017	0.000	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
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
0.000	1 000		0.000	0 007		0.400	0.000		1 405	0.000		0.000	4 455		-0.134	-0.415	0.491	3730
0.000	-1.009	1,175	0.000	-0.837		-0.458	0.000	1.742	0.173	0.000	0.000	0.000	1.155	0.000	-0.210	-1.254 -0.887	-0.487	3868
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
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	4319
0.000	0.815	-1.744	0.000	0.001	0.000	-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
0.000	0 568	1.036				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	0.000	1.000	0.000	0.337	0.000	2.011	0.000	1.040	0.220	0.000	0.000	0.000	0.000	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 5120
			0.000	-1.054	0.000	1.011	0.000	0.070	0.002	0.000	0.000	0.000	1.000	0.000	1.586	0.262	0.491	5128
															-1.277		-0.836	5201
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	5220 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 0.400	0.243	0.000	-2.564	0.000	0.507	0.000	0.941	-0.281	0.000	0.000	0.000	0.182	0.000	-0.365	-0.887	-0.040 -0.150	5858 5950
0.000	0.100	0.2.10				0.001	0.000	0.011	0.201	0.000	0.000	0.000	0.102	0.000	0.310	-4.000	-0.602	6175
																	0.000	6180
															-1.733	-1.379	-3.312	6182
						-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
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 0.262	0.491	7248
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	0 000	0 603	0 000	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	0.003	0.000	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	-0 565	0 000	0 000				-0.444	-1.634 -1.009	1.094	8019
			0.000	0.944	0.000	0.000	0.000	0.001	0.000	0.000	0.000				2.023	1.000	0.002	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 0.262	-0.040	8329 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
			0.000	-0.731	0.000	0.712	0.000	-0.514	0.349	0.000	0.000				-0.932	-1.254 0.905	-0.040 0.068	8626
0.000	-0.223	0.494	0.000	-0.033	0.000	0.7 12	0.000	0.01-1	0.040	0.000	0.000				0.594	-0.299	2.732	8663
															1.267	0.371	0.282	8742
0.000	0.815	0.039				0.507	0.000	-1,103	0.066	0.000	0.000	0.000	1,381	0.000	-0.603	-0.185	-0.602	8751
0.000	0.010	0.000				0.007	0.000		0.000	0.000	0.000	0.000		0.000	0.000	0.202	0.000	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	0 000	1 431	0 000	-0.300	0.000	-0 150	-0.281	0.000	0.000	0.000	0.436 1.381	0.000	-0.444	0.262	-0.487	8898
			2.000		0.000	0.112		5.100		0.000	0.000	0.000		0.000	5.210	0.410		8998
0.000	0.568	0.872	0.000	0.005	0.000	-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	U.UUU	-0.225	U.UUU										-0.058	1.913	-1.199	9524

Lab no.	b. Sample Suspected coliform bacteria (MF)			liform	Colif	orm bao	teria	Susp. t	hermo	tolerant	E.	coli (M	IF)	Colif	orm bac	teria	E. coli	("rapid'	' MPN)	
			bac	teria (N	1F)		(MF)		colifo	rm bao	:t. (MF)				("ra	apid" Ml	PN)			
	Α	вС	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С
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	32				0.329	0.151	0.275				0.046	0.000	-0.062						
n			0	0	0	60	60	59	0	C	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
Мах						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< th=""><th></th><th></th><th></th><th></th><th></th><th>2</th><th>1</th><th>0</th><th></th><th></th><th></th><th>0</th><th>0</th><th>1</th><th>1</th><th>1</th><th>3</th><th>2</th><th>0</th><th>0</th></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

_		-										T						
Presumptive C.			Clostridium			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count			Lab no.
perfringens (MF)			perfringens (MF)						1						22 °C, 3 days			
Δ	B	<u>``</u>	Δ	B	Ċ	Δ	B	0	Δ	B	<u> </u>	Δ	B	<u> </u>	Δ	B	<u></u>	1
	D	Ū	~	D	<u> </u>	~		<u> </u>	~		<u> </u>	~	-	<u> </u>	~	D	<u> </u>	
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
				05		40	10		40						70	=0	=-	
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 1 1 5 4	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
0.000	, 1.134	1.000	0.000	1.000	0.000	1.000	0.000	1.000	1.502	0.000	0.000	0.000	1.000	0.000	1.000	1.004	1.000	Sum
		-	-	-			_		-	_	_	-		_		-		Juin
() 0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	16
() 2	0	0	2	0	1	0	0	1	0	0	0	2	0	3	1	1	26
() 0	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0	2	18
() 1	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	11

Annex C – photos

10ml, 7 days



40 PT Microbiology – Drinking water, March 2018

100 ml, 7 days









42 PT Microbiology – Drinking water, March 2018

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