# **Drinking Water Microbiology** March 2022

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# Proficiency testing Drinking water Microbiology March 2022

### Parameters included

Coliform bacteria and *Escherichia coli* with membrane filter method (MF) Coliform bacteria and *Escherichia coli*, rapid kit-methods with MPN quantification *Clostridium perfringens* with MF Actinomycetes with MF Moulds with MF Yeasts with MF Culturable microorganisms (total count), 3 days incubation at 22±2 °C Slow-growing bacteria, 7 days incubation at 22±2 °C



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# **Abbreviations and explanations**

# Microbiological media

ACTA	Actinomycete Isolation Agar (according to SS 028212)
CCA	Chromocult Coliform Agar <sup>®</sup> (Merck; EN ISO 9308-1:2014)
Colilert	<sup>®</sup> Quanti-Tray <sup>®</sup> (IDEXX Inc.; EN ISO 9308-2:2014)
LES	m-Endo Agar LES (according to SS 028167)
m-FC	m-FC Agar (according to SS 028167)
R2A	Reasoner's 2 Agar (according to Standard Methods [5], 9215 Heterotrophic Plate Count)
RBCC	Rose Bengal Agar with both chlortetracycline and chloramphenicol (according to SS 028192)
TSC	Tryptose Sulfite Cycloserine agar (according to EN ISO 14189:2016)
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 nearest x-axis limit

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# General information on results evaluation

The proficiency testing program organised by the Swedish 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. As a result this report presents, for each parameter, method data where differences are present or could be expected.

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

Outliers and false results are not included in the calculation of mean value and measure of dispersion for the various method groups. The numbers of low and high outliers, as well as false results, are instead explicitly given in tables together with the group means etc. The mean and measure of dispersion are not shown for groups with four or fewer results, other 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 32 under "Processing of numerical results" with further reference to the scheme protocol [1].

# **Results of the PT round**

# **General outcome**

Test items were dispatched to 75 laboratories, 35 in Sweden, 33 in other Nordic countries (Faeroe Islands, Greenland and Åland included), two more from EU, and two from the rest of Europe and three from outside Europe. Results were reported by 69 laboratories.

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

Microorganisms and parameters of analyses are also compiled in table 1. For the MF analyses the parameters *suspected* coliform and thermotolerant coliform bacteria could be reported (shaded in tables 1 and 3). The results from suspected colonies are only used for interpretations and discussions, not for assessment.

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

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

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

Sample	Α			В			C		
Percentage of laboratories with         0       deviating results         1       deviating result         2       deviating results         >2       deviating results         >2       deviating results	6% 0 6%	% 88%		3% 3% 23% 7	21%		6% 1% 12%	31%	
No. of evaluable results	430	5		437			434	ł	
No. of deviating results $*$	12 (3	%)		27 (6 %	%)		19 (4	%)	
Microorganisms	Escherichia coli Hafnia alvei Clostridium perfr Phoma glomerata Sphingomonas sp	ringens a o.		Escherichia coli Klebsiella pneumo Clostridium biferm Streptomyces sp. Staphylococcus co. Sphingomonas sp.	niae nentans hnii		Cronobacter saka Enterobacter aer Phialophora mala Hanseniaspora u Pseudomonas flu	azakii ogenes orum varum orescer	9.5
Analysis	Target org.	F%	Х%	Target org.	F%	X%	Target org.	F%	Х%
Coliform bacteria (MF)	E. coli {H. alvei}	0	2	E. coli K. pneumoniae	0	2	C. sakazakii E. aerogenes	0	4
Susp. thermotolerant coliform bact. (MF)	E. coli	_	_	E. coli K. pneumoniae	-	-	C. sakazakii	_	-
E. coli (MF)	E. coli	0	4	E. coli	4	4	_	2	—
Coliform bacteria (rapid method)	E. coli {H. alvei}	0	0	E. coli K. pneumoniae	0	0	C. sakazakii E. aerogenes	0	2
E. coli (rapid meth.)	E. coli	0	0	E. coli	2	0	_	0	_
Presumptive C. perfringens (MF)	C. perfringens	0	0	C. bifermentans	13	0	-	3	—
C. perfringens (MF)	C. perfringens	8	0	[C. bifermentans]	25	-	_	0	_
Actinomycetes (MF) 25 °C	_	4	-	Streptomyces sp.	4	0	_	0	-
Moulds (MF) 25 °C	Ph. glomerata	0	6	_	6	-	Ph. malorum	23	0
Yeasts (MF) 25 °C	_	3	-	_	10	-	H. uvarum	0	13
Culturable micro- 22 °C organisms (total count), 3 days	(E. coli) (H. alvei) {Sphingo. sp.}	_	3	S. cohnii E. coli K. pneumoniae {Sphingo. sp.}	0	6	P. fluorescens C. sakazakii E. aerogenes	0	2
Slow-growing bacteria 22 °C (total count), 7 days	(E. coli) (H. alvei) (Sphingo. sp.)	-	3	Sphingo. sp S. cohnii E. coli K. pneumoniae	0	0	P. fluorescens C. sakazakii E. aerogenes	0	3

\* In total 35 of 69 laboratories (51 %) 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 primary cultivation media for the analysis of coliform bacteria were the enzymebased chromogenic medium CCA together with LES that is based on lactose fermentation (see p. 2 for media abbreviations). The group Other/Unknown with two laboratories include other media.

As in previous PT rounds, CCA gave lower average result than LES, here at least in sample B and C.

Madium	N			Α						В						С			
Medium	IN	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	48	46	18	20	0	1	0	47	290	11	0	1	0	45	2128	21	0	2	0
m-Endo Agar LES	22	22	19	21	0	0	0	22	313	8	0	0	0	21	2500	15	0	1	0
Chromocult C Agar	24	22	18	20	0	1	0	23	276	11	0	1	0	23	1904	22	0	0	0
Other/Unknown	2	2	_	_	0	0	0	2	-	_	0	0	0	1	-	_	0	1	0

In total six coliform bacteria, including E. coli, were present in the three samples.



#### Sample A

- Two strains of coliform bacteria were present in the sample, *Escherichia coli* and *Hafnia alvei*. *E. coli* grows with typical colonies, with a metallic sheen on LES and blue on CCA at 35/36/37 °C (see annex C). *H. alvei* has atypical colonies, red without a metallic sheen on LES and pale apricot pink on CCA (see annex C). Thus, the colonies of *H. alvei* should usually not be counted in as coliform bacteria on LES but should be included on CCA.
- The distribution of the results with a tendency of two peaks indicates differences in what has been counted in and not. Most of the results with ≤16 cfu/100 ml probably are results with *H. alvei* excluded. The dispersion (CV, see page 32) was just in between small and medium. One low outlier was reported.

#### Sample B

- Two strains of coliform bacteria, *E. coli* and *Klebsiella pneumoniae*, were present in the sample. These strains appeared with typical colonies at 35/36/37 °C, i.e. with metallic sheen on LES and blue and pink colonies, respectively, on CCA.
- The average result for coliform bacteria was higher with the rapid methods (p. 13) than with the MF-methods, 338 versus 290 cfu/100 ml, indicating that the coliform bacteria were not detected to the full extent by the MF-methods, particularly CCA. This is probably seen by the tail of lower results to the left of the main peak in the histogram. The reason for this is not clear.
- Despite the tail of lower results, the distribution was good with a small dispersion. One low outlier was reported.

#### Sample C

- Two strains of coliform bacteria were included, none of which was *E. coli*. Instead strains of *Enterobacter aerogenes* and *Cronobacter sakazakii* were present. Both strains show typical appearance on MF media at 35/36/37 °C, colonies with a metallic sheen on LES and with pinkish colours on CCA (see annex C).
- The distribution of the results was good with a small to medium dispersion. Two low outliers were present. They might be caused by mistakes in expressing the results for the volume 100 ml.
- The average recovery was much lower for the MF methods compared to for the rapid methods (2128 compared to 3103 cfu/100 ml; see p. 13), indicating that the coliform bacteria were detected to a lower extent by the MF-methods. This can be seen by the results ≤1400 cfu/100 ml in the histogram for the MF methods. It is obvious from the table and the histogram that more low results are attributed to the use of CCA compared to the use of LES.

# Suspected thermotolerant coliform bacteria (MF)

No evaluation in relation to performance is done for what is called suspected (i.e. not confirmed) colonies (see page 4). Therefore, no outliers are assessed. The *medians* are then more robust than the means and are given in the table and in histograms. **Thus, the parameter is not included in the performance assessment**.

The primary growth media at 44 or 44.5 °C used here to identify suspected thermotolerant coliform bacteria is m-FC. At least three of the laboratories in the group Other/Unknown have stated methods where the primary media are incubated at 35/36/37 °C, and where 44 °C is used only for confirmation. This is not the intention of the parameter suspected thermotolerant coliform bacteria according to the definition in the instruction and on the website for the program. It is the typical colonies appearing on the membrane filter at 44/44.5 °C that should be reported. Some laboratories have reported incubation at 44/44.5 °C, but where it is doubtful whether this really applies to the primary incubation.

Standard Mathad	N			Α			В				С		
Standard, Miethod	IN	n	Med	CV	$F \ < \ >$	n Med	CV	F < >	n	Med	CV	F	< >
Total	22	22	9	—		22 <b>287</b>	_		22	640	_	—	
SS 028167	9	9	11	_		9 289	-		9	1000	_	_	
SFS 4088	6	6	10	_		6 <b>295</b>	_		6	580	_	_	
NS 4792	2	2*	5	_		2* <b>190</b>	_		2*	40	_	_	
Other/Unknown	5	6	9	_		5 264	_		5	0	_	_	

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

\* Median given for comparison despite few results





#### Sample A

- The strain of *E. coli* appears as a suspected thermotolerant bacterium with blue colonies on m-FC at 44/44.5 °C. The strain of *H. alvei* should not be seen at 44 °C. Thus, the results are only reflecting *E. coli*.

#### Sample B

- Both the strain of *E. coli* and the strain of *K. pneumoniae* are growing as typical suspected thermotolerant coliform bacteria with blue colonies on m-FC agar at 44/44.5 °C.
- Five laboratories reported results that were more or less distant from the main peak.

#### Sample C

- Two coliform bacteria were included in the sample, of which the *C. sakazakii* strain appears as a suspected thermotolerant coliform bacterium; that is with grey-bluish colonies on m-FC at 44/44.5 °C. The strain of *E. aerogenes* may sometimes also grow as a suspected thermotolerant coliform bacterium with small blue colonies on m-FC, especially when the temperature does not fully reach 44 °C.
- The outcome is a bit strange. The results differs fairly much between laboratories and also between the stated reference standards. Six laboratories reported zero colonies while more than ten reported several thousands of colonies per 100 ml. It probably has to do a bit with what was growing at the temperature used. But more likely the main difference is how the methods are used. Are really all the suspected colonies on the primary media reported or only those that were positive also in a confirmation step at 44/44.5 °C? But as not all laboratories incubate their primary media at 44/44.5 °C the outcome will then nevertheless be incorrect. Either they report results from primary media incubated at 35/36/37 °C or final results after confirmation at the high temperature.

# Escherichia coli (MF)

Confirmation is required to identify and quantify *E. coli* when colonies are isolated from the primary cultivation media LES or m-FC. Depending on the method, tests for indole production and/or  $\beta$ -glucuronidase activity from oxidase-negative presumptive strains are usually performed. A violet to blue colony on CCA indicates positive  $\beta$ glucuronidase activity and is considered as a confirmed *E. coli*. Corresponding reactions occur on other chromogenic media based on  $\beta$ -glucuronidase activity.

The primary MF growth media here are CCA or LES used at 35/36/37 °C and m-FC used at 44/44.5 °C. In addition to the primary incubation temperature, the results are here also grouped by standard. CCA is stated by ISO 9308-1:2014 and should be incubated at 35/36/37 °C. For the standards from the Nordic countries (NS, SS and SFS) the majority of the results are from incubation at 35/36/37 °C on LES but some are also from incubation at 44/44.5 °C on m-FC.

There are no clear tendencies for any results versus incubation temperatures or methods. *E. coli* was included in sample A and B. The average results for LES could be a bit higher than the mean value for other MF methods incubated at 35/36/37 °C.

The dispersion (CV) is large in sample A for results where 44/44.5°C have been used. The dispersion is the largest for results by the Finnish standard in sample A and B.

Origin & Standard	NI#			Α						В						С			
Origin & Standard	19"	n	Mv	CV	F	<	<	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	>
Total	48	46	11	17	0	1	1	44	126	14	2	2	0	47	0	—	1	-	_
<u>Colony origin</u>																			
36 ± 2 °C	35	34	11	16	0	0	1	34	121	14	0	1	0	35	0	_	0	_	_
44/44.5 °C	6	5	11	26	0	1	0	5	121	12	0	1	0	6	0	_	0	_	_
36 ± 2 & 44/44.5 °C	7	7	9	16	0	0	0	5	171	11	2	0	0	6	0	_	1	_	_
<u>Standard</u>																			
ISO 9308-1:2014	27	25	10	16	0	1	1	23	119	12	2	2	0	27	0	_	0	_	_
SS 028167	10	10	14	9	0	0	0	10	140	10	0	0	0	10	0	_	0	_	_
SFS 3016 (4088)	8	8	11	21	0	0	0	8	131	17	0	0	0	8	0	_	0	_	_
"ISO 9308-1:1990"	2	2*	7	_	0	0	0	2*	160	_	0	0	0	1*	0	_	1	_	_
Other/Unknown	1	1	_	_	0	0	0	1	_	_	0	0	0	1*	0	_	0	_	_

All results

Results from the analysis of "coliform bacteria" MF at 35/36/37 °C

Madium	NT#			Α						В						С		
Medium	19"	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	< >
Total	39	37	11	16	0	1	1	35	121	13	2	2	0	39	0	_	0	
m-Endo Agar LES	13	13	13	13	0	0	0	12	128	13	0	1	0	13	0	_	0	
Chromocult C Agar	24	22	10	15	0	1	1	22	120	13	1	1	0	24	0	_	0	
Other/Unknown	2	2	_	_	0	0	0	1	_	_	1	0	0	2*	0	_	0	

# Compare the table above – the total number of results for 35/36/37 °C may differ somewhat due to different method information for coliform bacteria and *E. coli*.

\* Mean value is given for comparison despite few results



#### Sample A

- A strain of *E. coli* was included. It grows with typical colonies on the various primary growth media and expresses activity of  $\beta$ -glucuronidase and shows indole production as well as gas production.
- The distribution of the results was good and the average dispersion (CV) small. One low and one high outlier were reported.

#### Sample B

- One characteristic *E. coli* strain was included together with another coliform bacterium, *K. pneumoniae*. The colonies of both strains have characteristic metallic sheen on LES and are blue on m-FC. The *E. coli* strain is positive for  $\beta$ -glucuronidase activity, indole production and gas production. It forms blue colonies on CCA. *K. pneumoniae* is indole-negative, has no  $\beta$ -glucuronidase activity and have pink colonies on CCA, all these properties indicating that it cannot be mistaken for *E. coli* after confirmation.
- The distribution of the results was fairly good with a small dispersion. Two false negative results and two low outliers were present.

#### Sample C

- No *E. coli* was included but two other coliform bacteria, one strain of *E. aerogenes* and one strain of *C. sakazakii*. The strain of *C. sakazakii* is able to grow at 44 °C but *E. aerogenes* usually not. None of the strains are producing indole or  $\beta$ -glucuronidase. Thus, they cannot be mistaken for *E. coli*.
- One false positive result was reported.

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

The rapid method used for both these parameters was exclusively Colilert<sup>®</sup> Quanti-Tray<sup>®</sup> from the manufacturer IDEXX Inc. with incubation at 35, 36 or 37 °C. Of the 42 laboratories that reported Colilert some used trays with 51 wells, while others used trays with 97 wells. The laboratories often analysed both diluted and undiluted samples. Yellow wells (ONPG positive;  $\beta$ -galactosidase activity shown) will be interpreted as coliform bacteria and yellow wells also exhibiting fluorescence (MUG positive;  $\beta$ -glucuronidase activity shown) will be interpreted as *E. coli*.

Two laboratories used methods other than what was anticipated according to the instructions and are therefore allocated to the group Wrong method. They used ordinary MPN multiple tube methods,  $3 \times 5$  tubes, based on fermentation of substrate in the media followed by confirmation

When comparing results from different temperatures and maximum incubation times the differences are small and inconsistent. No grouping based on these criteria are therefore given.

There is a tendency for lower average results in all samples for both coliform bacteria and *E. coli* when using 51 wells instead of 97 wells. At the same time the dispersion (CV) is larger for 51 wells. The number of results from 51 wells is relatively low, which makes the comparison less certain.

There is no indication of interpretation difficulties in any sample. The results by the "Wrong methods" are included in the histograms; this is the reason for different total averages between the tables and the histograms.

Dringinlo	N			Α						В						С			
Principie	IN	n	Mv	CV	F	<	$^{\vee}$	n	Mv	CV	F	<	$^{\vee}$	n	Mv	CV	F	<	>
Total, Rapid meth.	42	42	15	24	0	0	0	42	341	12	0	0	0	39	3198	15	0	1	0
Colilert-18, 51 wells	9	9	10	23	0	0	0	9	<b>291</b>	21	0	0	0	7	2352	11	0	1	0
Colilert-18, 97 wells	33	33	16	22	0	0	0	33	355	11	0	0	0	32	3401	13	0	0	0
Wrong method <sup>#</sup>	2	2	_	_	0	0	0	2	_	_	0	0	0	2	_	_	0	0	0

Coliform bacteria, Rapid method with MPN

E. coli, Rapid method with MPN

Dringinlo	N			Α						В						С		
r meipie	14	n	Mv	CV	F	<	>	n	Mv	CV	F	<	$\vee$	n	Mv	CV	F	< >
Total, Rapid meth.	42	42	11	16	0	0	0	42	148	11	0	0	0	42	0	-	0	
Colilert-18, 51 wells	9	9	10	18	0	0	0	9	115	14	0	0	0	9	0	_	0	
Colilert-18, 97 wells	33	33	12	15	0	0	0	33	157	8	0	0	0	33	0	_	0	
Wrong method <sup>#</sup>	2	2	_	_	0	0	0	1	_	_	1	0	0	2*	0	_	0	

# No rapid kit method but a multiple tube method based on lactose fermentation

\* Mean value is given for comparison despite few results



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#### Sample A

- The strains of *E. coli* and *H. alvei* grow in the medium and possess the enzyme  $\beta$ -galactosidase. They should therefore be detected as coliform bacteria by methods based on this enzyme (ONPG positive) e.g. Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup>. The *E. coli* strain has the enzyme  $\beta$ -glucuronidase as well, and is therefore detected as *E. coli* by the MUG substrate. The activity of  $\beta$ -galactosidase is much weaker in *H alvei*; thus it usually necessary with 22 hours of incubation for that strain to be detected as a coliform bacterium.
- The distribution of *E. coli* was good with small dispersion, even though the distribution of coliform bacteria was a bit skewed with a tendency of a second peak with high results. That peak corresponds to results where *H. alvei* are included. But as the average for coliform bacteria is here still lower than by the MF methods (compare p. 6), it indicates that *H. alvei* has in many cases been excluded.

- No deviating result was identified, neither for coliform bacteria nor for E. coli.

#### Sample B

- Two different coliform bacteria, *E. coli* and *K. pneumoniae*, were included. Both strains possess the enzyme  $\beta$ -galactosidase and are detected as coliform bacteria. In contrast to *K. pneumoniae*, the *E. coli* strain also has the enzyme  $\beta$ -glucuronidase and hence is detected as *E. coli* as well.
- The distributions of both the coliform bacteria and *E. coli* results were good with small dispersions. One false negative result was reported for *E. coli*.
- The average result for coliform bacteria with the rapid methods was 17 % higher than the average result for MF methods (compare p. 6).

#### Sample C

- Two coliform bacteria, *E. aerogenes* and *C. sakazakii*, were included but no *E. coli*. Both strains are capable of growing in the medium and have the enzyme  $\beta$ -galactosidase and are detected as coliform bacteria. They lack the enzyme  $\beta$ -glucuronidase and hence are not detected as *E. coli*.
- The distribution of the results for coliform bacteria was good and the dispersion small. One low outlier was reported.
- The average here is about 50 % higher than by the MF method (see p. 6).

# Presumptive and confirmed *Clostridium perfringens* (MF)

The parameter *Clostridium perfringens* to be analysed is the sum of spores and vegetative cells of *C. perfringens*. The parameter is still included in the Drinking Water Directive of the European Union from December 2020 [7]. In Sweden it is accepted for laboratories to report colonies of presumptive *C. perfringens*, which is why that parameter is presented separately.

In new annexes [6] to the European Drinking Water Directive from 1998 [4] it is stated that the standard EN ISO 14189:2016, with its national editions, must be used after October 2017. The identical version ISO 14189 from 2013 can alternatively be used. Isolated colonies of presumptive *C. perfringens* on TSC agar are tested for acid phosphatase activity as confirmation for *C. perfringens*.

Before 2017 there was no international standard stated as reference method in the EU Directive [4]. A specific method was instead explicitly included into an original annex of the directive, the use of m-CP Agar incubated at 44 °C. That method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*. A draft standard, ISO/CD 6461-2:2002-12-20, based on TSC and confirmation with four different tests, was in parallel accepted as an alternative until a finished standard was available.

The majority of participants in this PT, 34 of 45 laboratories, used the standard (EN) ISO 14189. Only one laboratory stated the use of m-CP Agar, yet referring to ISO 14189:2013. Seven laboratories still claim the use of ISO/CD 6461-2:2002-12-20. *C. perfringens* was only present in sample A. *C. bifermentans* was present as presumptive *C. perfringens* in sample B. Knowing there are a lot fewer results, ISO/CD 6461-2 indicate higher averages than ISO 14189 in sample A but a lower average in sample B for presumptive *C. perfringens*.

Stondowd/Mathad	<b>N</b> T#			Α						В						С			
Standard/Method	IN	n	Mv	CV	F	<	>	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	<	$\vee$
Total	45	38	460	27	0	0	0	33	514	29	5	0	0	37	0	-	1	—	Ι
(EN) ISO 14189	34	30	442	30	0	0	0	26	549	28	4	0	0	29	0	_	1	_	Ι
ISO/CD 6461-2:2002	8	7	538	14	0	0	0	6	355	35	1	0	0	7	0	_	0	_	—
m-CP agar, EU-direct.	1	_	-	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
Other/Unknown	2	1	_	_	0	0	0	1	-	_	0	0	0	1*	0	_	0	_	_

Presumptive	Clostridium	perfringens	MF
		r - J - O - O	

Clostridium	perfringens	MF
	F - J - G	

Stondond/Mothed	<b>N</b> T #			Α						B					С			
Standard/Method	IN	n	Mv	CV	F	<	>	n	Mv	CV	F	< >	n	Mv	CV	F	<	>
Total	45	22	358	37	2	0	0	18	0	_	6		24	0	_	0	—	_
(EN) ISO 14189	34	15	301	46	2	0	0	11	0	_	6		17	0	_	0	_	_
ISO/CD 6461-2:2002	8	4*	516	16	0	0	0	4*	0	_	0		4*	0	_	0	_	_
m-CP agar, EU-direct	1	1	-	_	0	0	0	1*	0	_	0		1*	0	_	0	_	_
Other/Unknown	2	2	_	_	0	0	0	2*	0	_	0		2*	0	_	0	_	_

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

\* Mean value is given for comparison despite few results



#### Sample A

- A strain of *C. perfringens* was included. The colour of the colonies on TSC can vary from pale grey-brown to completely black, depending on the condition and reduction potential of the medium.
- No outlier was present in the presumptive test and two false negative result were present for *C. perfringens*.
- The distributions of the results were good for the majority of the results but with tails of lower results. The dispersions (CV) were therefore in average medium and large, respectively, for presumptive and confirmed C. *perfringens*.

#### Sample B

- A strain of *C. bifermentans* was included. The strain grows on TSC with fairly small, black to almost transparent presumptive colonies. Confirmation shows that the colonies are not *C. perfringens*.
- The dispersion was medium in average. Five false negative results were present for presumptive *C. perfringens*. Five false positive results were present in the analyses

of *C. perfringens*, indicating that either no confirmation was made or the confirmation results were misinterpreted.

#### Sample C

- No presumptive *C. perfringens* was included. Despite this, one false positive result was present for presumptive *C. perfringens*.

## Moulds and yeasts (MF)

Of the 31 laboratories that analysed moulds and yeasts, 23 reported that they followed the Swedish standard SS 028192. This standard is also partly used in Finland under their own national designation SFS 5507. Sometimes it is modified regarding media composition, for example dichloran (DRBC) can be used.

Various names are reported for the media linked to the use of SS 028192 and SFS 5507. These are: Cooke Rose Bengal agar base, Rose Bengal agar base, Rose Bengal agar, Rose Bengal Chloramphenicol agar and Dichloran Rose Bengal Chloramphenicol agar (DRBC). According to the original standard, dichloran should not be included in the medium (and thus DRBC should not be used) but instead Rose Bengal and the two stronger inhibitory substances chlortetracycline and chloramphenicol should be used.

Here, the media stated by the laboratories are shown, and a separation is made for those that used any form of "Rose Bengal agar" (RBC), DRBC, Malt Extract agar (ME) and Oxytetra Glucose Yeast Extract agar (OGYE). Four laboratories from various countries are stating DRBC in conjunction with SS 028192, SFS 5507 or "Standard methods" [5], which comprise the group DRBC "Water" in the tables. Only one laboratory stated this time NMKL 98:2005, modified to be used with DRBC, belonging to the group DRBC "Food" in the tables. ME agar was used by three Finnish laboratories and by one laboratory from Tanzania. No particular methods were stated for these four laboratories that comprise the group ME. One Finnish laboratory was

Standard Mathed	N			Α						В					С			
Standard, Miethod	IN	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	< >	n	Mv	CV	F	<	<
Total	31	29	14	12	0	0	2	29	0	_	2		24	515	21	7	0	0
<u>Medium</u>																		
RBC	20	19	14	10	0	0	1	19	0	_	1		18	481	18	2	0	0
DRBC "Water"	4	4*	12	14	0	0	0	3*	0	_	1		3*	563	30	1	0	0
ME	4	3*	14	4	0	0	1	4*	0	_	0		2*	495	14	2	0	0
DRBC "Food"	1	1	_	_	0	0	0	1*	0	_	0		1	_	_	0	0	0
OGYE	1	1	_	_	0	0	0	1*	0	_	0		0	_	_	1	0	0
Other/Unknown	1	1	_	_	0	0	0	$1^{*}$	0	_	0		0	_	_	1	0	0
<b>Magnification</b>														_				
None	14	13	15	10	0	0	1	13	0	_	1		10	529	12	4	0	0
1,1-4,9x	4	4*	15	14	0	0	0	3*	0	_	1		$4^{*}$	547	28	0	0	0
5-11,9x	12	11	13	13	0	0	1	12	0	_	0		10	<b>490</b>	28	2	0	0
16-19,9x	1	1	_	_	0	0	0	1*	0	_	0		0	_	_	1	0	0

\* Mean value is given for comparison despite few results

Yeasts MF

Standard Mathed	NT			Α				B					С			
Standard, Method	IN	n	Mv	CV F	< >	n	Mv	CV	F	< >	n	Mv	CV	F	<	>
Total	31	29	0	- 1		27	0	_	3		26	865	10	0	3	1
<u>Medium</u>																
RBC	20	20	0	- 0		19	0	_	1		19	895	10	0	0	1
DRBC "Water"	4	3*	0	- 0		$2^*$	0	_	1		3*	765	13	0	0	0
ME	4	3*	0	- 1		3*	0	_	1		$2^*$	802	8	0	2	0
DRBC "Food"	1	1*	0	- 0		1*	0	_	0		1	_	_	0	0	0
OGYE	1	1*	0	- 0		1*	0	_	0		1	_	_	0	0	0
Other/Unknown	1	1*	0	- 0		$1^{*}$	0	_	0		0	_	_	0	1	0
Magnification												_				
Ingen	14	13	0	- 1		13	0	_	1		11	810	9	0	3	0
1,1-4,9x	4	3*	0	- 0		3*	0	_	0		3*	788	7	0	0	0
5-11,9x	12	12	0	- 0		10	0	_	2		11	945	11	0	0	1
16-19,9x	1	1*	0	- 0		1*	0	_	0		1	_	_	0	0	0

\* Mean value is given for comparison despite few results



using an in house method with OGYE. The group Other/Unknown comprise only one laboratory using "Swedish medical standard". Several of the different groups contain so few results (<5) that discussion of possible differences related to media is not meaningful. The mean values are yet sometimes given for comparison.

No certain differences could be sorted out because of the few results in most groups. There seem to be no dependence between used magnification and result in any case.

#### Sample A

- No yeasts were included in the sample but the mould *Phoma glomerata*.
- The laboratory reporting the false positive yeast result also had deviating results for moulds and several other parameters, thus indicating a more general problem. Only one other high outlier ten times too much was present for moulds.
- The distribution of results of the moulds was good with a small dispersion.

#### Sample B

- Neither moulds nor yeasts were included. Despite this, two false positive results were reported for moulds and three for yeasts. The mould results were only 2 cfu per 100 ml, which probably could be attributed to down-fall from the laboratory air.

#### Sample C

- The mould *Phialophora malorum* and the yeast *Hanseniaspora uvarum* were included in quite similar concentrations. With the exception of the many zero results for moulds and the other deviating results, the result distributions were relatively good. The relative dispersion (CV) of the accepted results was medium for moulds and small for yeasts.
- There were 7 false negative results for moulds but none for yeasts, where there were one high and three low outliers instead.
- The false negative results for moulds are probably caused by the presence of small, undeveloped *P. malorum* colonies that were not interpreted as mould colonies. After seven days of incubation, they are often pink without mature spores (see the photo in annex C).

### Actinomycetes (MF)

Actinomycetes is a prescribed parameter for drinking water monitoring according to Swedish regulations, and therefore mainly Swedish laboratories perform this analysis. It is performed according to the Swedish standard for actinomycetes in water, SS 028212 (1994). Five Finnish laboratories performed the analysis based on other methods, and are placed in the group Other together with a Swedish laboratory that did not use the ordinary Swedish medium. Notably, the Finnish laboratories stated the use of natamycin as the selective substance instead of cycloheximide, and they counted actinomycetes after 14 days and often after 7 days as well. The base agar medium varied within this group, but all laboratories used other media than Actinomycete Isolation Agar (ACTA) that is the base medium in the Swedish standard.

The averages of the two groups ACTA and Other in sample B are equal. However, the dispersion (CV) was smaller within the group Other.

Madimm/Standard	N			Α					В						С		
Medium/Standard	IN	n	Mv	CV	F	< >	n	Mv	CV	F	<	$^{\prime}$	n	Mv	CV	F	< >
Total	27	26	0	-	1		26	29	10	1	0	0	27	0	-	0	
ACTA (SS 028212)	21	20	0	_	1		21	29	10	0	0	0	21	0	_	0	
Other	6	6	0	_	0		5	29	4	1	0	0	6	0	_	0	



#### Sample A

- No actinomycetes was included in the sample. One false positive result was reported.

#### Sample B

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

#### Sample C

- The sample contained no actinomycetes and there were no false positive results.

## Culturable microorganisms 22 °C, 3 days

Sixty-three of the 66 laboratories performing the analysis stated the use of EN ISO 6222:1999, which prescribes Yeast extract Agar (YEA). While still stating the use of EN ISO 6222:1999, seven laboratories used Plate Count Agar (PCA) instead. Two laboratories used YEA in conjunction with Standard methods [5] and the last one YEA together with an unknown method (no method stated). These three laboratories comprises the group "Other method". The majority of the laboratories claimed counting both bacterial and fungal colonies. Six laboratories stated that they did not count fungi, and two stated that they counted yeasts but not moulds.

Since all but three 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 the magnification at reading.

As usual, it is difficult to find any consistent method differences both for media and magnification. In both sample B and C, Plate Count Agar gave, as often before, lower result than YEA, while the dispersion (CV) was higher. In sample C, with highest colony concentration, the average was the lowest where no magnification was used for reading.

Course of manufac	N		Α	В	С
Group of results	IN	n	Mv CV F < >	n Mv CV F < >	n Mv CV F < >
Total, all results	66	64	<b>0</b> 159 0 0 2	62 <b>29</b> 13 0 1 3	65 <b>82</b> 20 0 1 0
EN ISO 6222	63	61	0 160 0 0 2	59 <b>29</b> 13 0 1 3	62 <b>81</b> 20 0 1 0
Medium					
Yeast extract Agar	56	54	0 153 0 0 2	52 <b>30</b> 11 0 1 3	55 <b>85</b> 17 0 1 0
Plate Count Agar	7	7	<b>0</b> 265 0 0 0	7 <b>25</b> 22 0 0 0	7 <b>52</b> 32 0 0 0
Magnification					
None	14	14	<b>0</b> 214 0 0 0	12 <b>28</b> 13 0 0 2	14 <b>71</b> 27 0 0 0
1,1–4,9×	21	19	0 102 0 0 2	20 <b>29</b> 15 0 1 0	20 86 16 0 1 0
5–11,9×	28	28	0 208 0 0 0	27 <b>30</b> 11 0 0 1	28 <b>82</b> 19 0 0 0
Other method	3	3*	0 - 0 0 0	$3^*$ 24 - 0 0 0	$3^* 102 - 0 0 0$

\* Mean value is given for comparison despite few results





#### Sample A

- The colonies mainly consisted of *E. coli* and *H. alvei* but some laboratories probably also counted one or two colonies of *Sphingomonas* sp.
- The distribution of the results was acceptable, even though there were many zero results due to the very low average (< 1 cfu/ml). Because of the low average the dispersion (CV) was very high, but here without any implication. Two high outliers were present.

#### Sample B

- The colonies mainly consisted of a strain of *S. cohnii* but colonies of the coliform bacteria were also present, and perhaps also some colonies of *Sphingomonas* sp.
- The distribution of the results was very good with a small dispersion. One low outlier and three high outliers were present.
- The high outliers could be caused by inclusion of colonies of the slow-growing strain of *Sphingomonas* sp., which could be seen under magnification from at least day four of incubation.

#### Sample C

- The colonies mainly consisted of a strain of *P. fluorescens* but colonies of the two coliform bacteria were also present.
- The distribution of the results was a bit broad but still good. The dispersion was just in between small and medium. It has been seen before that the strain of *P*. *fluorescens* often gives a bit dispersed results. One low outlier was present.

# Slow-growing bacteria 22 °C, 7 days

Thirty-seven laboratories performed the analysis of slow-growing bacteria. The parameter is mandatory to monitor according the Swedish Drinking water ordinances, and therefore a custom method is adopted and used by the Swedish laboratories. Before 2003 a Swedish standard was available, but today a modified version of the method in the standard EN ISO 6222:1999 is used, which prescribes incubation on Yeast extract Agar (YEA). The modifications include: incubation at  $22\pm1$  °C for seven days, using magnification when reading the plates (at least 4×, preferentially 10×), and that only bacterial colonies must to be counted.

There is a process ongoing within ISO to develop a standard method for the parameter "slow-growing microorganisms". The current proposal is to use a more nutrient depleted medium than YEA, namely "Reasoner's 2 Agar" (R2A) and spread-plate technique instead of pour-plate technique. In this PT, R2A was used by six laboratories and they constitute a separate group in the table and the histograms, four of which were the only laboratories also stating the spread-plate technique.

Twenty-two laboratories stated they do not include fungal colonies when present, while 11 stated that they include both moulds and yeasts. Another four laboratories stated that they include yeasts but not moulds.

According to the table beneath, there might be an effect of magnification upon the number of colonies counted in sample B and C. However, there are always other factors present as well, like media and inoculation technique, implying it is difficult to state exactly what the real cause of differing results is. Although there were only four results by spread-plating, they gave 12-13 % lower average result than by pour-plating in sample B and C (figures not shown).

For sample A, the few laboratories that used R2A on average reported lower results than those that used YEA. However, these differences are probably not due to the medium, but rather to the magnification that was used when counting the colonies. This is obvious since the difference is even more pronounced when comparing different degrees of magnification.

In both samples A and C there are no apparent differences in recovery between culturable microorganisms and slow-growing bacteria. Contrary to this, for sample B the average number of slow-growing bacteria is 15 times more abundant than culturable microorganisms. Only this sample contained a decent number of slow-growing bacteria and the broad distribution with a tendency to two peaks illustrates the problem with the outcome. These colonies are often small and therefore difficult to count without magnification, in particular with pour-plating, which probably explains the difference in recovery between the laboratories for sample B.

Most Swedish laboratories normally use YEA and  $10 \times$  magnification. This is probably the explanation for the fairly equal and relatively high average results for YEA as well as the magnification 5–11.9×.

Choup of nogult	N			Α						В						С			
Group of result	IN	n	Mv	CV	F	<	<	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	37	36	1	97	0	0	1	37	432	27	0	0	0	35	86	18	1	1	0
<u>Medium</u>																			
Yeast extract Agar	30	30	1	90	0	0	1	30	435	26	0	0	0	29	88	16	0	1	0
"Reasoner's 2 Agar"	7	7	<1	128	0	0	0	7	420	32	0	0	0	6	75	24	1	0	0
<u>Magnification</u>																			
None	3	3*	<1	_	0	0	0	3*	215	_	0	0	0	3*	61	_	0	0	0
1.1–4.9×	7	6	<1	113	0	0	1	7	469	27	0	0	0	5	80	24	1	1	0
5–11.9×	25	25	1	86	0	0	0	25	442	26	0	0	0	25	89	16	0	0	0
$\geq$ 12×	1	1	_	_	0	0	0	1	_	_	0	0	0	1	_	_	0	0	0
Unknown	1	1	_	_	0	0	0	1	_	_	0	0	0	1	_	_	0	0	0

\* Mean value is given for comparison despite few results



#### Sample A

- Almost no colonies were growing. There might be some individual ones of the slow-growing *Sphingomonas* sp. or of coliform bacteria and possibly yeasts.
- The outcome was quite similar to the one of culturable microorganisms; the average was also here very low, around 1 cfu/ml, with a high dispersion of the results. The many zero results were fully acceptable. One high outlier was present.

#### Sample B

- The result distribution for the slow-growing bacteria in the sample was fairly good. However, a tail of lower results was present that could be seen as a second smaller peak. The average dispersion was yet medium.
- The first "peak" around 200 cfu/ml represents results were only a part of the "slow growing" *Sphingomonas* sp. seems to have been counted. However, many more colonies have there been counted than the culturable microorganisms *S. cohnii* and the coliform bacteria. The colonies of *Sphingomonas* sp. are often small also after seven days of incubation, in particular when the pour-plate technique has been used. The ease to detect colonies is of course partly affected by the magnification used. Yet this is not clear from the results, probably because there are only few results in some magnification groups.
- No deviating results were present due to the fairly large (medium) dispersion.

#### Sample C

- No specific slow-growing bacteria were present in the sample. Thus, the same strains of bacteria as for culturable microorganisms were present also after 7 days of incubation. Thus, the majority consisted of *P. fluorescens* and coliform bacteria.
- The distribution of the results was similar to that of culturable microorganisms, as was also the dispersion. One false negative result and one low outlier were present.

# Outcome of the results and laboratory assessment

# General information about reported results

The distributions of results for the respective analysis are shown in the 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 analysis in the summarising 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 of an individual laboratory can be broadly assessed by the numbers of false results and numerically high z-scores (absolute values), including the outliers.

Generally, the laboratories that did not report their results in due time need to evaluate their results themselves. This can be done by comparison with the results of all other laboratories, by looking in tables, figures and annex A.

# Mixed up results and other practical errors

Eleven laboratories have more than one deviating result. Laboratory 3076 seem to have mixed up two samples and the corresponding sample numbers are crossed out in annex A. A number of laboratories seem to have performed incorrect calculations from their colony readings, for one or more results, to the final concentration of 100 ml. One laboratory reported four false positive results and some other deviating results as well for specific analyses, which indicate misunderstanding regarding the analysis or reporting, or contamination of the samples in their own laboratory.

# 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 are not further evaluated here. They are given explicitly to facilitate the follow-up process for laboratories using z-scores in control charts etc. For interpretation and calculation of z-scores, see the explanation to annex A and the scheme protocol [1].

The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better the agreement is 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'.s
- The outliers are included in the plots after recalculation to "z-scores" with the same standard deviation (s) as the rest of the results for each parameter. The outliers are also included in No. of results.
- *z*-scores > +4 and < -4 have in the plots been set to +4 and -4, respectively.
- The numbers of false positive and false negative results are given in the table under the plots together with the numbers of outliers.
- *The horizontal line in each box indicates the median for the laboratory.*
- The box includes 25 % of the results above and below the median. The lines protruding from the box and/or the circles embrace the remaining 50 % of the results, false results excluded.
- A circle is for technical reasons shown when a result is to a certain degree deviating\* from the rest. This alone does not mean it is an outlier.
- The background is divided into coloured fields to simplify localization of the laboratory results.
- \* < [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**

The round comprised three test items with different microorganism compositions. The test material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd [2]. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition and approximate concentrations in the samples obtained at the Swedish 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 current European Drinking water directive [4] and its updates [6], as well as the new directive that is under implementation [7]. Alternative methods and other standards can usually be used without any problem.

Sample <sup>1</sup>	Microorganisms	Strain co	llection no.	<b>cfu/100 ml</b> <sup>2</sup>
		SLV (own)	Reference <sup>3</sup>	
A	Escherichia coli	165	CCUG 43600	13
	Hafnia alvei	015	CCUG 45642	18
	Clostridium perfringens	442	CCUG 43593	530
	Phoma glomerata	543	CBS 119226	14
	Sphingomonas sp.	547	CCUG 36955	4 *
В	Escherichia coli	084	From water	170
	Klebsiella pneumoniae	186	CCUG 45102	210
	Clostridium bifermentans	009	CCUG 43592	690
	Streptomyces sp.	548	From water	27
	Staphylococcus cohnii	462	CCUG 35411	26*
	Sphingomonas sp.	547	CCUG 36955	$670$ $^{*}$
С	Cronobacter sakazakii	419	From water	1600
	Enterobacter aerogenes	099	ATCC 13 048	1300
	Phialophora malorum	545	From water	800
	Hanseniaspora uvarum	555	CF SQE 77 $^{\#}$	1000
	Pseudomonas fluorescens	535	CCUG 45106	110 *

**Table 2** Microorganisms present in the samples

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

2 cfu = colony forming units; \* indicates cfu per ml; concentrations at latest check

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 that has not yet been typed at another culture collection

# Designation of an older culture collection

# Quality control of the test material

In order to allow comparison of results from the freeze-dried samples, it is essential that the original sample mixture is homogeneous and that a uniform volume is distributed in all vials. The sample volume was monitored during production by weighing 2-3 % of the vials before and after addition of the sample. The largest detected differences between vials were 9, 5 and 9 mg in samples 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 samples; shaded rows are not used for performance assessing

Analysis parameter				Sa	mple	<sup>1</sup>			
Method standard for analysis		Α			B			С	
	cfu	I2	Т	cfu	I2	Т	cfu	I <sub>2</sub>	Т
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	30 <sup>a</sup>	1.6	1.6	38 <sup>b</sup>	0.7	1.3	29 <sup>a</sup>	0.4	1.2
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	6	_	_	36 <sup>b</sup>	0.8	1.3	6 <sup>b</sup>	2.1	2.8
Escherichia coli (MF) m-Endo Agar LES according to SS 028167	13	1.5	2.0	17 <sup>b</sup>	0.6	1.4	—	_	_
Presumptive Clostridium perfringens (MF) TSC Agar according to SS-EN ISO 14189:2016	53 <sup>b</sup>	2.4	1.5	69 <sup>b</sup>	0.9	1.3	—	_	_
Moulds (MF) Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192	14	0.5	1.5	—	_	_	8ª	1.3	2.2
Yeasts (MF) Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192	_	_	_	_	_	_	10 <sup>a</sup>	0.7	1.7
Actinomycetes (MF) Actinomycete Isolation Agar with cycloheximide according to SS 028212	_	_	_	27	1.9	1.7	—	_	_
Culturable microorg., 3d 22 °C (pour plate) Yeast extract Agar according to SS-EN ISO 6222:1999	<1	_	_	30	1.4	1.5	110	3.9	1.5
Slow-growing bacteria, 7d 22 °C (pour plate) Reasoner's 2 Agar acc. to Standard Methods [5] Process acc. to SS-EN ISO 6222:1999 modified	4	1.97	4.1	70°	0.9	1.3	—	_	_

1 10 vials for sample A and B and 5 vials for sample C analysed in duplicate, normally100 ml for MF and 1 ml for pour plate, analysed 16, 14 and 15 weeks prior the testing round for the sample A, B and C, respectively

a Determined for the volume 1 ml

b Determined for the volume 10 ml

c Determined for the volume 0.1 ml

- No target organism and thus no analysis

Table 3 shows the results from the organizer in the form of concentration means (cfu) and the measures (I<sub>2</sub> and T; see reference 1) used to assess homogeneity. The values are from duplicate analyses of 10 vials the first time a sample mixture is used or from duplicate analyses of 5 vials when a sample mixture is used a second time. The results relate to the volume that was used for counting the colonies. The criterion used for a sample mixture to be considered homogeneous is that I<sub>2</sub> and T *not simultaneously* are higher than 2. According to that criterion, all sample mixtures were homogeneous with regard to the parameters that were to be analysed and assessed. However, the result of the suspected thermotolerant coliform bacteria in sample C (here *C. sakazakii*), that should not be assessed and thus not matters, indicated inhomogeneity.

### Processing of numerical results

Most histograms have "tails" in either or both directions, due to values that do not belong to the expected distribution. For drinking water, log<sub>10</sub> transformation of the results is normally not routine. Instead, for the low concentrations normally encountered here, square root transformations of the results usually give the best normal distributions by decreasing the significance of the high deviating results. Very deviating values will still be 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 the 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, for example when many zero results are reported and in some borderline cases, subjective adjustments are made based on the knowledge of the sample mixture's content in order to set the correct limits. 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 here 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 uncertainties will be expressed as a square root values. 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. It is here provided as the relative uncertainty ( $u_{rel}$ ), which is expressed as per cent after division by the mean value mv and multiplication by 100.

More information about result processing and recommendations on follow-up analyses are provided in the scheme protocol [1]. A pdf of that document is available on the websites <u>https://www2.slv.se/absint</u> and <u>http://www.livsmedelsverket.se/en/pt-micro</u>.

# References

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

Lab no.	Sample	Suspec	ted co	iform	Coliform	bacter	ria (MF)	Susp. th	ermoto	olerant	Ε.	coli (M	F)	Colifo	orm bac	teria	E. coli	("rapid"	MPN)
	ABC	bact	teria (M	F)	•	в		colifor	m bact.	. (MF)	٨	в		("ra	pid" MF	<u>(N</u>	۸	B	
1131	3 1 2	<u> </u>	-	-	- -	-	-	-	-	-	<u> </u>	-	-	- -	-	-	-	-	-
1237	3 1 2	-	-	-	1	44	-	-	-	-	1	14	<1	3	100	-	3	64	<1
1545	321	32	310	2400	32	310	2400	14	300	1200	14	140	0	6	475	3218	6	122	0
1611	213 213	20	3/3	2400	20	3/3	2400	15	284	- 080	8	207	-	21	387 293	3065	20	152	0
1868	3 2 1	26	317	3175	26	317	3175	-	-	-	16	135	0	27	348	4253	14	162	0
1970	123	8	330	3000	8	330	3000	8	330	0	8	110	0	-	-	-	-	-	-
2317	123	-	-	-	14	189	1450	-	-	-	14	119	0	-	-	-	-	150	-
2745	3 1 2	20	300	1100	20	300	1100	20	300	1100	10	180	0	-	- 340	2900	-	- 150	-
3055	321	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
3145	231	-	-		-	-	-	-	-	-	-	-	-	17	365	2909	17	1/4	0
3162	3 1 2	_	-		_		-	_	-	-	-	-	-	10	291	4106	10	155	0
3305	2 1 3	22	260	2800	22	260	2800	-	-	-	13	130	<100	15	330	5200	15	110	<1
3730	2 1 3	12	240	1100	-	-	-	11	150	600		-	-	-	-	-	-	-	-
3883	312	27	345	3200	27	345	3200	-	-	-	14	144	<1	18	330	2800	11	208	<1
4015	3 2 1	-	-		-		-	-	-	-	-		-	- 20	430	4300	- 10	- 105	-
4339	321	-	-	-	8	300	2500	5	290	1600	8	120	0	11	490	2400	11	120	0
4343	321	-	-	-	-	-	-	-	-	-	-		-	12	411	5475	12	157	0
4356 4419	321	25	350	2400	25 8	350 160	2400	13	300	480	13 g	74	0	13	488 240	2419 020	13	172	0
4723	1 3 2	-	-	- 000	-	- 100	- 500	-	-	-	-	-	-	43	345	2420	18	186	0
4889	1 2 3	-	-	-	22	270	1700	-	-	-	9	80	0	12	310	4400	12	130	0
5018	231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5201 5220	213	-			- 15	321 285	1855	-		-	32	146	0	-		-	-	-	-
5321	3 1 2	-	-	-	8	270	2180	-	-	-	8	125	ŏ	4	350	2300	4	130	0
5333	231	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5352	231	-	-	-	18	340	2500	6	280	0	6	140	0	-	-	-	-	-	-
5447 5553	1 2 3 1	-	-	-	14	225	- 1310	-	-	-	14	130	<1.0	-	-	-	-	-	-
5701	1 2 3	-	-	-	23	330	1740	-	-	-	20	150	0	-	-	-	-	-	-
5858	231	-	-	-	13	230	920	-	-	-	7	106	0	-		-	-	-	-
5950 6175	312	25	364	3400	25	364	3400	8	289	1300	11	118	<1	17	326	>2419	17	179	<1
6175	$\frac{2}{123}$	-	-	-	-	-	-	-	-	-	-	-	-	7.5	340	2537	7.5	00.5 174	<1
6233	2 3 1	7	160	1750	7	160	1750	-	-	-	7	0	0	8	228	2851	8	140	0
6253	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	20	320	3000	12	150	0
6421 6449	231	-	-	-	- 16	250	- 22	-	-	-	- 7	-	- 1	-	-	-	-	-	-
6456	1 2 3	_	-		10	200	1900	_		-	5	80	0	12	384	2117	12	137	0
6563	1 3 2	19	264	3700	19	264	3700	19	264	3700	15	90	<1	23	349	3964	10	214	<1
6686	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	8.7	340	1500	8.7	120	<1
7248	321	15	360	1100	15	360	1100	90	460	590	15	200	<1	13	365	3870	13	170	<1
7688	2 1 3	9	300	2800	9	300	2800	_	-	-	9	20	ő	9	330	5000	9	120	0
7728	2 1 3	-	-	-	7	330	1700	-	-	-	7	130	0	-	-	-	-	-	-
7876	231	12	330	2100	12	330	2100	5	260	1000	12	140	0	13.4	324.8	2330	13.4	164.3	0
7930 7962	123	18	340	2500	18	340	2500	-		-	10	95	0	12	340	2500	12	140	0
7968	2 3 1	20	330	2400	20	330	2400	20	330	0	8	160	0	12	357	4840	12	171	0
8068	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8260 8329	321	10	276	1203	15	276	1247	-	-	-	10	124	0	- 25	315	- 3764	- 12	-	-
8359	1 3 2	-	-		17	222	1309	-	-	-	7	82	- <1	- 25		- 3704	- 12	- 155	-
8435	2 1 3	-	-	-	20	160	600	3	140	80	10	70	0	-	-	-	-	-	-
8569	2 1 3	-	-	-	24	356	2700	-	-	-	13	157	0	24	387	3080	12	157	0
8626	213	9	220	130	21	220	130	9	45 240	0	7	220	39	-		-	-	-	-
8663	1 3 2	26	310	3800	26	310	3800	6	310	1400	18	160	0	18	370	- 2400	9	140	0
8742	2 1 3	-	-	-	20	330	1900	-	-	-	9	150	<1	-	-	-	-	-	-
8751	123	-	-	-	-	-	-	-	-	-	-	-	-	11	207	2220	11	87	<1
8829	3 1 2	- 13	- 340	980	- 13	- 340	980	8	350	1700	- 13	140	-	15	308	∠900 -	15	- 100	-
8862	1 2 3	36	354	3200	36	354	3200	-	-	-	16	172	0	22	342	3445	14	145	0
8898	1 2 3	27	318	3108	27	318	3108	-	-	-	10	116	0	18	367	3873	5	146	0
8955	1 3 2	- 10	262	2800	25	340	3000	6	280	450	15	140	0	27	260	3300	15	140	0
9524	1 2 3	-	203	2000 -	22	203	2500	-	200	- 102	8	80	0	13	399	3130	13	127	0
9736	321	-	-	-	-	-	-	-	-	-	-	-	-	9	365	3759	6	150	0
9899	1 3 2	24	338	4000	24	338	4000	-	-	-	13	138	0	26	360	2677	18	144	0
9903 Mean	231	29	400	2842	29	400 290	2842 2128	12	342	2091	12	133	0	- 15	338	3103	- 11	- 147	-
CV (%)					20	11	21				17	14	-	25	12	16	18	11	-

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

Presu	umptive	e C.	Clo	ostridiu	m	Мо	ulds (Mi	F)	Ye	asts (MI	F)	Actino	nycetes	(MF)	Total	plate co	unt	Slow-gro	owing b	acteria	Lab no.
perfri	ngens	(MF)	perfri	ingens	(MF)	•									22 °C	C, 3 day	s#	22 °	<u>C, 7 day</u>	/s <sup>#</sup>	
A -	<u>-</u>	· ·	A .	- B	- -	A -	<u>в</u> -	<u> </u>	A -	<u>в</u> -	<u> </u>	A -	<u>в</u> -	<u> </u>	A .	<u>в</u> -	<u> </u>	A -	<u>в</u> -	- -	1131
-	-	-	10	20	<1	-	-	-	-	-	-	-	-	-	<1	12	10	-	-	-	1237
732	650	0	732	0	0	9	0	327	0	230	1000	0	19	0	0	36	82	3	490	84	1545
564	- 818	-	-		-	- 19	-	264	- 0	-	- 782	- 0	- 25	-	0	40	83 101	1	565 600	91 105	1611 1753
767	1014	Ő	-	-	-	-	-	-	-	-	-	Ő	35	Ő	Ő	29	126	1	632	126	1868
370	430	0	370	0	0	11	0	0	0	0	720	-	-	-	0	14	87	-	-	-	1970
119	0	0	-	1	1	-	-	-	-	-	-	-	-	-	< 10	25	85	-	-	-	2317
-	-	-	440	-	-	-	-	-	-		-	-	-	-	0	30	48	0	- 152	- 51	2037
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	299	61	-	-	-	3055
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65	20	120	75	600	4	3076
-	-	-	-	-	-	-	-	-		-	-	-	-	-	4	434	120	-	-	-	3145
760	340	0	-	-	-	10	0	600	0	0	900	0	30	0	0	17	114	2	490	114	3162
590	300	<1	590	<1	<1	14	<1	<1	<1	<1	91	<1	27	<1	<1	30	110	-	-	-	3305
473	710	- <1	473	710	- <1	- 15	- <1	480	- <1	- <1	772	<1	26	- <1	<1	39	143	<1	103	143	3730
541	514	0	-	-	-	18	0	333	0	0	775	0	27	0	0	41	106	2	632	100	4015
-	-	-	-	-	-		-	-	-	-	-	-	-	-	0	26	111	-	-	-	4288
555	0	-	550	0	0	17	2	1000	-	-	-	0	24	0	1	39	67 129	15	560 770	75 137	4339
490	460	0	490	0	0	-	-		-	-	- 503	-	-	-	1	31	50	-		-	4356
40	320	1800	40	320	0	32	0	0	90	260	710	-	-	-	0	29	76	-	-	-	4418
460	712	0	- ⊿10	-	-	11	0	273	0	0	1000	0	40	0	2	37 27	114 00	3	480	108	4723
-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5018
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	289	86	5201
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	40	30	1	215	50	5220
-	-	-	-		-	-	-	-			-	-	-	-	<1	31	43 67	-		-	5333
470	690	0	470	0	0	11	0	460	0	0	600	0	30	0	0	32	52	0	187	54	5352
-	-	-	-		-	15	0	600	0	0	10	0	27	0	0	29	94	0	590	30	5447
-	-	-	420	<1,0	<1,0	-	-	-	-		-	-	-	-	- 1	32	71	-		-	5553 5701
-	-	-	450	520	0	-	-	-	-	-	-	-	-	-	1	30	133	-	-	-	5858
536	<1	<1	536	<1	<1	9	<1	545	<1	<1	800	<1	32	<1	<1	32	108	2	580	111	5950
-	-	-	-		-	-	-	-	-		-	-	-	-	0 <1	20	135 49	- <1	-	- 50	6175 6182
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	23	138	-	-	-	6233
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<10	34	130	-	-	-	6253
-	-	-	-	-	-	- 16	-	-	-	-	-	-	- 20	-	- 2	- 26	-	-	-	-	6421 6448
-	-	-	-	-	-	-	-	-	-	-		-	- 25	-	1	38	86	-	-	-	6456
155	636	<1	-	-	-	15	<1	464	<1	1	818	9	35	<1	<1	23	46	<1	64	46	6563
360	<1 (130)	<1	-	-	-	- 10	-	1	-	-	-	-	- 20	-	<1	48 18	81	-	-	- 51	6686 7248
932	430 695	0	-		-	-	-		-	-	- 1500	0	29	0	0	32	66	2	532	69	7442
69	70	0	35	35	0	14	0	450	0	0	690	0	30	0	0	22	110	-	-	-	7688
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	24	44	-	420	-	7728
710	49	0	710	0	0	160	0	1000	0	0	900	-	- 24	-	0	25	55	-	420	-	7930
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7962
580	156	0	580	0	0	24	0	0	0	0	690	-	-	-	1	36	58	-		-	7968
475	0	0	-	-	-	-	-	-	-	-	-		-	-	1	26	100	2	- 575	- 107	8260
1000	700	0	-	-	-	11	0	320	0	0	1073	0	21	0	0	34	94	0	630	91	8329
-	-	-	196	<1	<1	-	-	-	-	-	-	-	-	-	3	22	89	-	-	-	8359
404	100	0	-	-	-	-	-	-			-	-	-	-	20	33	111	4	500	127	8569
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	23	40	-	-	-	8626
520	730	0	520	0	0	15	0	1200	0	0	936	-	-	-	5	41	68	5	470	77	8628
470	- 130	-	-	- 130	-	-	-	-	-		-	-	-	-	1	32 29	92 64	-		-	8742
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	33	116	<10	495	95	8751
450	672	0	-	-	-	12	0	800	0	0	990	0	28	0	0	28	49	0	176	65	8766
573	- 718	-	- 573	-	-	14	-	- 336	-	-	- 981	0	25	-0	0	185	- 84	<1 1	70 500	<1 103	8829
482	649	õ	-	-	-	14	2	427	0	Ő	856	-	-	-	0	32	120	6	460	121	8898
300	620	0	300	0	0	13	0	400	0	0	900	0	33	0	0	29	110	<10	560	120	8955
509 44	673 85	0	44	-	-	13	0	0	0	0	855	0	31	0	2	20 31	61 84	1	/55	/6	9436 9524
509	856	õ	-	-	-	15	0	591	0	0	1018	0	45	0	0	22	104	1	704	104	9736
671	905	0	-	-	-	13	0	448	0	0	986	0	25	0	0	32	78	4	550	79	9899
433	850 514	0	359	-	-	18	0	442 515	0	0	2392	0	35	0	0	36	128	1	614 432	126	9903 Moan
27	29	-	37	-	-	12	-	21	-	-	10	-	10	-	159	13	20	97	27	18	CV (%)

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E.	coli (M	F)	Colif ("ra	orm bac apid" Mi	teria PN)	E. coli ("rapid" MPN)			
	ABC	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	Α	В	С	
n		28	28	28	47	48	47	22	22	22	48	48	48	44	44	42	44	44	44	
Min		7	160	130	1	44	23	3	0	0	1	0	0	3	100	200.5	3	0	0	
Мах		36	400	4000	36	400	4000	90	460	3700	32	220	39	43	490	5475	20	214	0	
Median		20	317.5	2450	19.5	310	2400	8.5	286.5	640	10	130	0	13	345	3000	12	150	0	
Mean					18	290	2128				11	126	0	15	338	3103	11	147	0	
CV (%)					20	11	21				17	14	-	25	12	16	18	11	-	
False po False pe	sitive				0	0	0				0	0	1	0	0	0	0	0	0	
Outliers.	low				1	1	2				1	2	ŏ	Ő	0	1	ő	0	Ő	
Outliers,	high				0	0	0				1	0	0	0	0	0	0	0	0	
Low limi	t OK	7	160	130	7	160	500	3	0	0	5	70	0	3	100	920	3	64	0	
High lim	it OK	36	400	4000	36	400	4000	90	460	3700	20	220	0	43	490	5475	20	214	0	
					1 4 6 4 4	17.010	10.100	-			0.050	11.000	0.000	0.007	10.001	FF 700	0.017	10 100	0.000	
mv (√Mean)	)				4.244	17.018	46.130				3.258	11.229	0.000	3.827	18.394	55.703	3.317	12.132	0.000	
s (CV*mv/*	100)				0.865	1.840	9.730				0.550	1.583	0.000	0.954	2.187	9.087	0.603	1.337	0.000	
<b>u</b> <sub>rel,mv</sub> (% (100*s/ v	<b>′∂)</b> ∕n <sub>mv</sub> /mv)				3.0	1.6	3.1				2.5	2.1		3.8	1.8	2.5	2.7	1.7		
x (√Resuli	t)																			
<b>z</b> ([x-mv]/s,	)																			

# cfu/ml

Presumptive C. perfringens (MF)			CI perfi	lostridiu ringens	m (MF)	Moulds (MF)			Yeasts (MF)			Actino	mycete	s (MF)	Tota 22 °	plate c C, 3 da	ount ys <sup>#</sup>	Slow-g 22	Lab no.		
Α	В	С	Α	В	С	Α	В	C	Α	В	С	Α	В	С	Α	В	С	Α	В	С	
				0.1	0.4	0.4	0.4	0.1				07	07	07				07	07		1
38	38	38	24	24	24	31	31	31	30	30	30	27	27	27	66	66	66	37	37	37	n Min
1000	1014	1800	732	730	0	160	2	1200	90	260	2392	9	45	0	65	434	143	75	770	143	Max
			102		•		-	.200	00	200	2002	Ű		Ū	00						mux
495	649	0	460	0	0	14	0	457.5	0	0	855.5	0	28.5	0	0	30.5	84	1	500	91	Median
460	514	0	358	0	0	14	0	515	0	0	865	0	29	0	0	29	82	1	432	86	Mean
27	29	-	37	-	-	12	-	21	-	-	10	-	10	-	159	13	20	97	27	18	CV (%)
0	0	1	0	6	0	0	2	0	1	3	0	1	0	0	0	0	0	0	0	0	False pos.
0	5	0	2	0	0	0	0	/ 0	0	0	0	0	1	0	0	1	1	0	0	1	Faise neg.
0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	2	3	0	1	0	0	Outliers >
-	-	-	-	-	-	_	-	-	-	-	-	-	-	-	_	-	-		-	-	
40	49	0	10	0	0	9	0	264	0	0	600	0	19	0	0	12	10	0	64	30	Low limit
1000	1014	0	732	0	0	24	0	1200	0	0	1500	0	45	0	5	48	143	15	770	143	High limit
-																					
21.439	22.678	0.000	18.932	0.000	0.000	3.718	0.000	22.704	0.000	0.000	29.416	0.000	5.383	0.000	0.385	5.399	9.032	0.970	20.785	9.282	mv
5.782	6.640	0.000	7.083	0.000	0.000	0.431	0.000	4.863	0.000	0.000	2.954	0.000	0.512	0.000	0.611	0.676	1.793	0.943	5.530	1.632	S
4.4	5.1		8.0			2.2		4.4			2.0		1.9		19.8	1.6	2.5	16.2	4.4	3.0	u <sub>rel.mv</sub> (%)
																					x
																					z

Lab no.	Sample	Suspected coliform	Colif	orm bac	teria	Susp. thermotolerant	E. coli (MF)			Colif	orm bac	teria	E. coli	' MPN)	
	ABC	bacteria (MF) A B C	А	(MF) B	с	coliform bact. (MF)	Α	в	с	("ra	apid" Mi B	PN) C	А	в	с
1131	3 2 1							_	-					_	-
1237	123		-3.750 1.634	-4.000 0.320	0 204		-4.000	-4.000 0.381	0.000	-2.196	-3.837 1.555	0 113	-2.630	-3.091	0.000
1611	3 1 2		0.989	1.247	0.294		-0.781	1.995	0.000	-0.381	0.585	-1.226	0.245	0.147	0.000
1753	3 1 2									0.792	-0.584	-0.038	1.918	-0.193	0.000
1868 1970	132 312		0.989	0.428	1.050		1.348	0.246	0.000	1.435	0.119	1.047	0.705	0.446	0.000
2317	3 1 2		-0.580	-1.777	-0.827		0.878	-0.203	0.000						
2637	1 3 2		0.004	0.405	4 000		0.475	4 200	0.000	-0.535	0.021	-0.204	0.000	0.087	0.000
2745 3055	2 1 3 3 2 1		0.264	0.105	-1.332		-0.175	1.382	0.000						
3076	123														
3145	213									0.310	0.325	-0.195	1.338	0.792	0.000
3162	3 1 2									-0.697	-0.610	0.922	-0.256	0.238	0.000
3305	123		0.517	-0.485	0.697		0.631	0.109	0.000	0.048	-0.104	1.806	0.923	-1.230	0.000
3730	3 1 2 3 1 2		1.102	0.846	1.073		0.878	0.487	0.000	0.436	-0.104	-0.307	0.000	1.713	0.000
4015	3 1 2									1.333	1.289	1.252	-0.256	0.534	0.000
4288	213		-1.636	0 165	0 308		-0 781	-0 174	0 000	-0 535	1 711	-0 730	0.000	-0.881	0 000
4343	2 1 3		1.000	0.100	0.000		0.701	0.114	0.000	-0.381	0.859	2.013	0.245	0.298	0.000
4356	3 1 2		0.875	0.919	0.294		0.631	-1.659	0.000	-0.232	1.690	-0.718	0.480	0.735	0.000
4418	231		-1.036	-2.3/4	-2.443		-0.781		0.000	2.862	-1.327	-0.716	1.537	1.127	0.000
4889	213		0.517	-0.318	-0.504		-0.469	-1.443	0.000	-0.381	-0.360	1.170	0.245	-0.546	0.000
5018 5201	3 1 2			0489	-0 315		4 000	0 530	0 000						
5220	3 1 2		-0.428	-0.074	-0.145		-1.113	-0.468	0.000						
5321	1 3 2		-1.636	-0.318	0.058		-0.781	-0.031	0.000	-1.915	0.144	-0.852	-2.185	-0.546	0.000
5333 5352	312 312		-0.001	0 772	0.398		-1 470	0.381	0 000						
5447	1 3 2		0.001	0.112	0.000		1.470	0.001	0.000						
5553	231		-0.580	-1.096	-1.021		0.878	0.109	0.000						
5701	231		-0.738	-1.006	-0.454		-1.113	-0.590	0.000						
5950	3 1 2		0.875	1.120	1.252		0.106	-0.232	0.000	0.310	-0.155		1.338	0.933	0.000
6175 6182	213									-1.141	-1.936	-4.000	-0.959	-2.038	0.000
6233	1 3 2		-1.848	-2.374	-0.442		-1.113		0.000	-1.047	-1.506	-0.254	-0.810	-0.224	0.000
6253	321									0.676	-0.231	-0.103	0.245	0.087	0.000
6421 6448	231 321		-0 282	-0 655	-4.000		-1 113	-1 808	0 000						
6456	3 1 2		-0.580	-1.562	-0.261		-1.857	-1.443	0.000	-0.381	0.550	-1.067	0.245	-0.320	0.000
6563	1 3 2		0.133	-0.418	1.511		1.117	-1.101	0.000	1.015	0.132	0.799	-0.256	1.868	0.000
7248	321		-0.428	1.063	-1.332		1.117	1.840	0.000	-0.920	0.325	0.716	0.480	0.678	0.000
7442	321		0.875	0.274	-0.194		0.631	0.643	0.000	1.123	1.343	-0.581	0.480	1.817	0.000
7688 7728	123		-1.438	0.165	0.697		-0.469	-4.000	0.000	-0.867	-0.104	1.652	-0.525	-0.881	0.000
7876	3 1 2		-0.901	0.624	-0.031		0.374	0.381	0.000	-0.175	-0.170	-0.818	0.571	0.513	0.000
7930	321		-0.001	0.772	0.398		-0.175	-0.936	0.000	-0.381	0.021	-0.628	0.245	-0.224	0.000
7962	2 3 1		0.264	0.624	0.294		-0.781	0.897	0.000	-0.381	0.229	1.526	0.245	0.707	0.000
8068	321														
8260 8329	123		-0.428	-0.220	-1.112		-0.175	-0.059	0.000	1 220	0 083	0 622	0 245	-0 448	0 000
8359	231		-0.139	-1.151	-1.023		-1.113	-1.373	0.000	1.223	0.000	0.022	0.240	0.440	0.000
8435	312		0.264	-2.374	-2.224		-0.175	-1.808	0.000	1 400	0.505	0.000	0.045	0.000	0.000
8626	∠ 3 1 2 1 3		-1.438	-1.187	-3.569		-1.113	0.822 2.276	0.000	1.123	0.565	-0.023	0.245	0.298	0.000
8628	132		0.392	-0.485	-0.761		-1.113	-1.101	0.000						
8663 8742	312		0.989	0.320 0.624	1.595		1.788	0.897 0.643	0.000	0.436	0.385	-0.739	-0.525	-0.224	0.000
8751	3 1 2		0.20-1	0.024	0.201		0.400	0.040	0.000	-0.535	-1.832	-0.945	0.000	-2.098	0.000
8766	123		-0.738	0.772	-1.524		0.631	0.381	0.000	0.048	-0.386	-0.204	0.923	0.387	0.000
8862	321		2.031	0.977	1.073		1.348	1.191	0.000	0.905	0.046	0.329	0.705	-0.068	0.000
8898	123		1.102	0.443	0.989		-0.175	-0.290	0.000	0.436	0.349	0.719	-1.793	-0.037	0.000
8955 9436	312 132		0.875	0.772	0.697		1.117	0.381 -0.621	0.000	-0.697	-1.037	0.192	0.923	-0.224 1.424	0.000
9524	1 2 3		0.517	-0.829	0.398		-0.781	-1.443	0.000	-0.232	0.723	0.027	0.480	-0.645	0.000
9736	3 1 2		0.750	0 743	1 750		0.624	0 207	0.000	-0.867	0.325	0.617	-1.439	0.087	0.000
9903	312		1.320	1.621	0.738		0.031	0.327	0.000	1.333	0.200	-0.430	1.03/	-0.099	0.000
n		0 0 0	47	40	47	0 0 0	40	16	47	A A	A A	40	A A	40	A A
Min		U U U	-3.750	48 -4.000	-4.000	0 0 0	48 -4.000	40 -4.000	47 0.000	-2.196	-3.837	42 -4.000	-2.630	43 -3.091	44 0.000
Max			2.031	1.621	1.759		4.000	2.276	0.000	2.862	1.711	2.013	1.918	1.868	0.000
Median			0.133	0.297	0.058		-0.175	0.109	0.000	-0.232	0.083	-0.149	0.245	0.087	0.000
Mean			-0.080	-0.083	-0.161		0.000	-0.174	0.000	0.000	0.000	-0.095	0.000	0.000	0.000
30			1.130	1.140	1.247		1.280	1.2/9	0.000	1.000	1.000	1.100	1.000	1.000	0.000
z<-3			1	1	2		1	2	0	0	1	1	0	1	0
-3≤z<-2 2 <z≤3< th=""><th></th><th></th><th>0</th><th>3</th><th>2</th><th></th><th>0</th><th>0</th><th>0</th><th>1</th><th>0</th><th>1</th><th>2</th><th>2</th><th>0</th></z≤3<>			0	3	2		0	0	0	1	0	1	2	2	0
z>3			0	0	Ő		1	0	0	0	0	0	0	Ő	0

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

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

Presumptive C. Clostridium perfringens (MF) perfringens (MF)					Mo	oulds (N	NF)	Yeasts (MF)			Actinomycetes (MF)			Tota 22	l plate c °C. 3 da	ount	Slo bacteri	Lab no.			
A	B	C	A	B	() C	Α	В	С	Α	В	С	Α	В	С	A	B	C	A	<u>в</u>	C	
0.971 0.399 1.082 -0.381 -1.821	0.424 0.892 1.380 -0.292	0.000 0.000 0.000 0.000 0.000	-2.226 1.147 0.043 0.289	0.000 0.000 0.000	0.000 0.000 0.000 0.000	-1.666 1.488 -0.931	0.000 0.000 0.000	-0.950 -1.328	0.000	0.000	0.747 -0.492 -0.875	0.000	-1.999 -0.748 1.040	0.000	-0.630 -0.630 -0.630 -0.630 -0.630 -0.630 -0.630 -0.630 -0.630 <b>4.000</b> <b>2.643</b>	-2.860 0.889 1.369 0.382 -0.020 -2.450 0.382 0.116 4.000 -1.370 4.000	-3.273 0.013 0.044 0.568 1.223 0.165 0.105 0.456 -1.173 -0.681 -4.000 1.072	0.808 0.032 1.092 0.032 -1.029 <b>4.000</b>	0.244 0.540 0.671 0.787 -1.529 0.671	-0.072 0.158 0.591 1.190 -1.311 <b>-4.000</b>	1131 1237 1545 1611 1753 1868 1970 2317 2637 2745 3055 3076 3145
1.060 0.493 0.054 0.315 0.367 0.121	-0.638 -0.807 0.598 -0.001	0.000 0.000 0.000 0.000 0.000	0.756 0.398 0.638 0.452	0.000 0.000 0.000	0.000 0.000 0.000 0.000	-1.289 0.056 0.361 1.219 0.941 0.655	0.000 0.000 0.000 0.000 0.000	0.368 -0.163 -0.916 1.834 -0.282	0.000 0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000 0.000	0.198 -4.000 -0.552 -0.534 -1.604	0.000 0.000 0.000 0.000 0.000 0.000	0.184 -0.365 -0.555 -0.365 -0.945	0.000 0.000 0.000 0.000 0.000 0.000	-0.630 -0.630 -0.630 -0.630 -0.630 -0.630 1.007 1.007	-1.886 0.116 -1.047 1.251 1.485 -0.443 1.251 1.011 0.250	0.918 0.812 -0.018 1.632 0.705 0.839 -0.472 1.297 -1.093	0.471 -1.029 -1.029 0.471 0.471 <b>3.078</b>	0.244 0.816 -1.923 0.787 0.521 1.259	0.855 -0.173 1.640 0.440 -0.381 1.484	3155 3162 3305 3730 3883 4015 4288 4339 4343 4356
-2.614 0.002	-0.721 0.603	0.000	0.186	0.000	0.000	<b>4.000</b> -0.931	0.000	-1.271	0.000	0.000	-0.938 0.747	0.000	1.838	0.000	-0.630 1.685 1.007 -0.630 1.007 -0.630	-0.020 1.011 -0.300 1.369 -4.000 0.250 0.282	-0.175 0.918 0.254 -1.982 -1.380 -0.472	0.808 -1.029 0.032	0.203 -0.684 -1.107	0.680 -0.005 -1.354	4418 4723 4889 5018 5201 5321 5333 5333
0.296	0.541	0.000	0.388 0.221 0.322 0.596	0.000	0.000 0.000 0.000 0.000	-0.931 0.361 -1.666	0.000	-0.236 0.368 0.132	0.000	0.000	-0.383	0.000	0.184 -0.365 0.534	0.000	-0.630 -0.630 1.007 1.007 -0.630 -0.630 -0.630	0.382 -0.020 0.382 0.116 0.382 -1.370 0.639 -0.891	-0.338 1.395 0.759 1.443 -1.133 1.514	-1.029 -1.029 0.471 -1.029	0.634 0.596 -1.811	-1.104 -2.331 0.768 -1.354	5352 5447 5553 5701 5858 5950 6175 6182 6233
-1.555 -0.426 0.529 1.572 <b>-2.271</b> 0.121	0.383 -0.292 0.555 <b>-2.155</b> 0.117	0.000 0.000 0.000 0.000 0.000	-1.838		0.000	0.655 0.361 -1.289 0.056	0.000 0.000 0.000 0.000	-0.239 -0.307	0.000 0.000 0.000 0.000	0.000 0.000 0.000	-4.000 -0.276 3.153 -1.066	0.000 0.000 0.000 0.000	0.004 1.040 0.004 -0.179 0.184	0.000 0.000 0.000 0.000 0.000	-0.630 1.685 1.007 -0.630 -0.630 -0.630 -0.630 -0.630 -0.630 1.007	0.639 -0.443 1.132 -0.891 <b>2.261</b> -1.709 0.382 -1.047 -0.739	1.322 -0.506 0.135 -1.254 -0.018 -1.833 -0.506 0.812 -1.337 -1.254	-1.029 -1.029 0.471	-2.312 -0.524 0.412	-1.531 -1.311 -0.597	6253 6421 6448 6456 6563 6686 7248 7442 7688 7728 7728
0.901	-2.361	0.000	1.089 0.727	0.000	0.000	4.000	0.000	1.834	0.000	0.000	0.198	0.000	0.010	0.000	-0.630 1.007	-0.590 0.889	-0.901	0.111	0.000		7930 7962 7968
0.062 1.761 0.159 -0.232	0.569 0.117 -1.909	0.000 0.000 0.000 0.000	-0.696	0.000	0.000	-0.931	0.000	-0.990	0.000	0.000	1.131	0.000	-1.563	0.000	1.007 -0.630 <b>2.205</b> <b>4.000</b> -0.630 -0.630 <b>3.030</b>	-0.443 0.639 -1.047 0.511 0.511 -0.891 1.485	0.540 0.370 0.224 0.074 0.839 -1.510 -0.438	0.471 -1.029 1.092	0.578 0.780 0.285	0.651 0.158 1.218	8068 8260 8329 8359 8435 8569 8626 8628
-0.039	0.654	0.000	0.017	0.000	0.000	-0.588	0.000	1.148	0.000	0.000	0.693	0.000	-0.179	0.000	-0.630 1.007 1.007 -0.630	0.382 -0.020 0.511 -0.159	0.312 -0.575 0.970 -1.133	-1.029 -1.029	0.265	0.285 -0.747	8663 8742 8751 8766
0.432 0.089 -0.712 0.194 <b>-2.561</b> 0.194	0.620 0.421 0.335 0.492 <b>-2.027</b> 0.991	0.000 0.000 0.000 0.000 0.000 0.000	0.707 -0.228 -1.736	0.000 0.000 0.000	0.000 0.000 0.000	0.056 0.056 -0.260 -0.260 0.361	0.000 0.000 0.000 0.000	-0.899 -0.419 -0.556 0.330	0.000 0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000 0.000	0.645 -0.054 0.198 -0.060 0.843	0.000 0.000 0.000 0.000	-0.748 0.705 0.360 <b>2.586</b>	0.000 0.000 0.000 0.000	-0.630 -0.630 -0.630 1.685 1.685 -0.630	<b>4.000</b> 0.382 -0.020 -1.370 0.250 -1.047	0.074 1.072 0.812 -0.681 0.074 0.650	-1.029 0.032 1.569 -1.029 0.032 0.032	-2.246 0.285 0.120 0.521 1.210 1.039	0.531 1.053 1.025 -0.346 0.561	8829 8862 8898 8955 9436 9524 9736
0.772 -0.109 38	1.115 0.976 33	0.000 0.000 37	22	18	24	-0.260 1.219 31	0.000 0.000 29	-0.316 -0.345 24	0.000 0.000 29	0.000 0.000 27	0.672 4.000 30	0.000 0.000 26	-0.748 1.040 26	0.000 0.000 27	-0.630 -0.630 66	0.382 0.889 66	-0.112 1.273 66	1.092 0.032 37	0.482 0.722 37	-0.241 1.190 36	9899 9903 n
-2.614 1.761 0.140 0.000 1.000	-2.361 1.380 0.421 0.000 1.000	0.000 0.000 0.000 0.000 0.000	-2.226 1.147 0.355 0.000 1.000	0.000 0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000 0.000	-1.666 4.000 0.056 0.258 1.390	0.000 0.000 0.000 0.000 0.000	-1.328 2.455 -0.270 0.000 1.000	0.000 0.000 0.000 0.000 0.000	0.000 0.000 0.000 0.000 0.000	-4.000 4.000 -0.060 -0.267 1.731	0.000 0.000 0.000 0.000 0.000	-1.999 2.586 -0.088 0.000 1.000	0.000 0.000 0.000 0.000 0.000	-0.630 4.000 -0.630 0.121 1.203	-4.000 4.000 0.250 0.121 1.381	-4.000 1.632 0.074 -0.061 1.108	-1.029 4.000 0.032 0.108 1.185	-2.312 1.259 0.285 0.000 1.000	-4.000 1.640 0.076 -0.111 1.190	Min Max Median Mean SD Sum
0 3 0 0	0 3 0 0	0 0 0	0 1 0 0	0 0 0	0 0 0	0 0 1 2	0 0 0	0 0 1 0	0 0 0	0 0 0	3 0 0 2	0 0 0	0 0 1 0	0 0 0	0 0 2 3	1 2 1 3	2 0 0 0	0 0 0 2	0 2 0 0	1 1 0 0	16 20 11

# Annex C – photos







100 ml

10 m



40 PT Microbiology – Drinking water, January (March) 2022



No fungi in the sample!

No photo available!





10 ml

100 ml



PT Microbiology – Drinking water, March 2020

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# PT reports published 2021

Proficiency Testing – Food Microbiology, January 2021, by Jonas Ilbäck

- Proficiency Testing Drinking Water Microbiology, March 2021, by Linnea Blom and Tommy Šlapokas
- Proficiency Testing Food Microbiology, April 2021, by Jonas Ilbäck
- Proficiency Testing Drinking Water Microbiology, September 2021, by Linnea Blom and Tommy Šlapokas
- Proficiency Testing Food Microbiology, October 2021, 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 Swedish Food Agency's PT program offers

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

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

## The Swedish Food Agency's reference material

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

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