

Drinking Water Microbiology

March 2015

by Tommy Šlapokas



Edition

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Proficiency testing
Drinking water Microbiology
March 2015



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**

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² Laboratory work

Abbreviations and explanations




Microbiological media

LES	m-Endo Agar LES (according to SS 028167)
LTTC	m-Lactose TTC Agar with Tergitol (acc. to EN-ISO 9308-1:2000)
m-FC	m-FC Agar (acc. to SS 028167)
PAB/TSC/SFP	Tryptose Sulfite Cycloserine Agar (acc. to ISO/CD 6461-2:2002)
RBCC	Rose Bengal Agar with both chlortetracycline and chloramphenicol (acc. to SS 028192)
YeA	Yeast extract Agar (acc. to EN ISO 6222:1999)
CCA	Chromocult Coliform Agar [®] (Merck; EN ISO 9308-1:2014)
Colilert	Colilert [®] Quanti-Tray [®] (IDEXX Inc.; EN ISO 9308-2:2014)

Other abbreviations

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

Legend to method comparison tables

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

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General information on results evaluation

The histograms and calculation of outliers are described on page 28 under "Processing of numerical results" with further reference to the scheme protocol (1).

The proficiency testing program organised by the National Food Agency is accredited against EN ISO/IEC 17043. This standard prescribes that results should be grouped based on the method used. Therefore it is mandatory for participants to inform about method data. 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 no consistency between the standard referred to and the information given regarding various method details. Results from laboratories with ambiguous details are either excluded or placed in the group "Other/Unknown" in the tables, together with results from methods used only by individual laboratories.

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

Results of the PT round March 2015

General outcome

Test items were sent to 99 laboratories, in Sweden 40, in other Nordic countries (Faeroe Islands and Åland included) 48, from EU 3, from the rest of Europe 2 and from countries outside Europe 6. Results were reported from 95 laboratories.

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

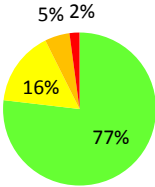
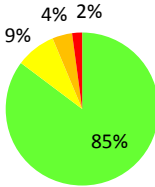
Microorganisms and parameters of analyses are also compiled in **table 1**. For the MF analyses the parameters *suspected* coliform bacteria and thermotolerant coliform bacteria on primary media could be reported as well. The results from suspected colonies are only used for interpretations and discussions.

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

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

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

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

Mixture	A			B			C		
Percentage of laboratories with									
No. of evaluable results	564			562			565		
No. of deviating results *	22 (4%)			18 (3%)			14 (2%)		
Microorganisms	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Phialophora malorum</i> <i>Kluyveromyces maraxianus</i> <i>Staphylococcus xylosus</i>			<i>Escherichia coli</i> <i>Citrobacter freundii</i> <i>Clostridium perfringens</i> <i>Pseudomonas putida</i>			<i>Clostridium perfringens</i> <i>Hanseniaspora uvarum</i> <i>Kluyveromyces maraxianus</i> <i>Streptomyces sp.</i>		
Analysis	Target	F%	X%	Target	F%	X%	Target	F%	X%
Coliform bacteria (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	3	4	<i>E. coli</i> <i>C. freundii</i>	1	3	–	1	–
Susp. thermotolerant coliform bact. (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	–	–	<i>E. cloacae</i> { <i>C. freundii</i> }	–	–	–	–	–
<i>E. coli</i> (MF)	<i>E. coli</i>	5	3	<i>E. coli</i>	1	0	–	0	–
Coliform bacteria (rapid method)	<i>E. coli</i> <i>K. pneumoniae</i>	0	5	<i>E. coli</i> <i>C. freundii</i>	0	8	–	0	–
<i>E. coli</i> (rapid meth.)	<i>E. coli</i>	0	3	<i>E. coli</i>	0	7	–	0	–
Presumptive <i>C. perfringens</i> (MF)	–	11	–	<i>C. perfringens</i>	0	4	<i>C. perfringens</i>	2	4
<i>Clostridium perfringens</i> (MF)	–	3	–	<i>C. perfringens</i>	0	3	<i>C. perfringens</i>	3	11
Actinomycetes (MF) 25 °C	–	–	–	–	3	–	<i>Streptomyces sp.</i>	6	6
Moulds (MF) 25 °C	<i>Ph. malorum</i>	0	0	–	2	–	–	0	–
Yeasts (MF) 25 °C	<i>K. maraxianus</i>	0	0	–	5	–	<i>H. uvarum</i> <i>K. maraxianus</i>	0	–
Culturable micro-organisms (total count), 3 days 22 °C	<i>S. xylosus</i> <i>K. maraxianus</i> (<i>E. coli</i>) (<i>K. pneumoniae</i>)	0	10	<i>P. putida</i> (<i>E. coli</i>) (<i>C. freundii</i>)	1	5	<i>Streptomyces sp.</i> (<i>H. uvarum</i>) (<i>K. maraxianus</i>)	2	4

* In total 38 of 95 laboratories (40%) reported at least one deviating result

– Organism missing or numerical result irrelevant

() The organism contributes with only very few colonies

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

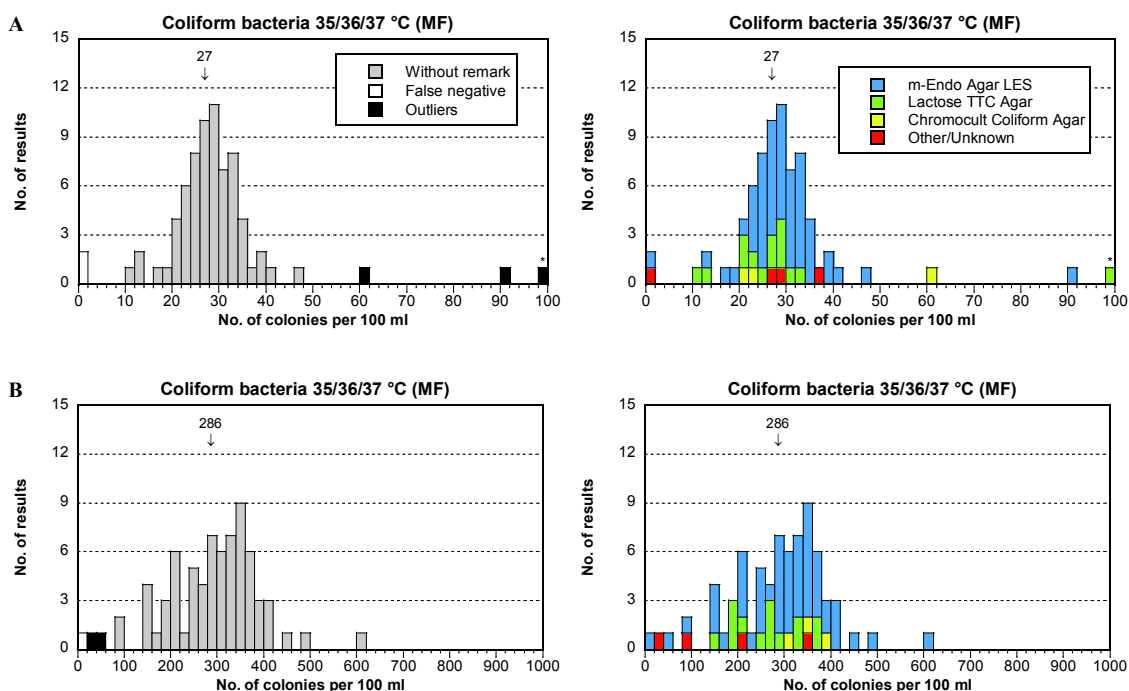
{ } The organism may give different results depending on method or definition used

Coliform bacteria (MF)

In two cases (both from Iceland) the primary medium reported was not the one prescribed in the standard referred to. Here we have assumed the medium to be correct. The medium Endo Agar reported by some participants is here included in m-Endo Agar LES (LES).

From the table it is clear that LES was almost four times more frequently used than LTTC. There is an indication that LTTC, as often, gave a somewhat lower mean result compared to LES. However, for the relative dispersion is no tendency seen. Individual deviating results was obtained within all groups.

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	73	68	27	12	2	0	3	70	286	17	1	2	0	72	0	–	1	–	–
m-Endo Agar LES	52	50	28	11	1	0	1	50	298	16	1	1	0	52	0	–	0	–	–
Lactose TTC Agar	14	13	23	15	0	0	1	14	255	14	0	0	0	13	0	–	1	–	–
CCA	3	2	21	–	0	0	1	3	339	–	0	0	0	3	0	–	0	–	–
Other/Unknown	4	3	30	–	1	0	0	3	192	34	0	1	0	4	0	–	0	–	–



Mixture A

- The strains of *E. coli* and *K. pneumoniae* grow with typical colonies, a metallic sheen on LES and yellow on LTTC at 37 °C. The relative dispersion of the results was small.

- The average recovery is well in accordance with that for coliform bacteria with the rapid methods (see page 12).

Mixture B

- This mixture has about 10 times more coliform bacteria than mixture A. The strains of *E. coli* and *C. freundii* grow as typical suspected coliform bacteria, a metallic sheen on LES and yellow on LTTC at 37 °C. Three low deviating results were obtained. There is also a tendency of too many low results and, thus, skewness to the left, in comparison with the rapid method (see page 13).
- The average recovery of coliform bacteria in this mixture was somewhat lower than with the rapid methods.

Mixture C

- No coliform bacteria were included in the mixture and no false positive results were present.

Suspected thermotolerant coliform bacteria (MF)

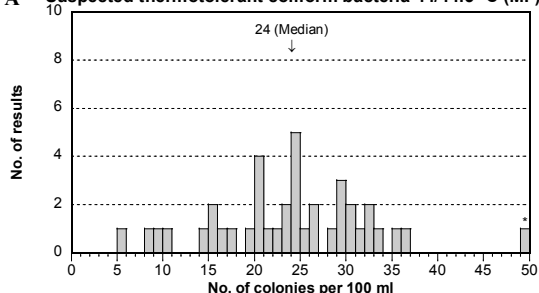
The two most used growth media were m-FC and LTTC. The incubation temperature was 44 or 44.5 °C. Here, results were separated based on the method standards most commonly used to get a further division beyond the media. They are EN ISO 9308-1:2000 with LTTC and three standards with m-FC from the Nordic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

Thermotolerant coliform bacteria are not equally defined in all countries. Due to this, only the parameter suspected thermotolerant coliform bacteria is shown, which *is not included in performance assessment*. The table shows the *medians* instead of mean values because outliers are not identified for parameters called suspected.

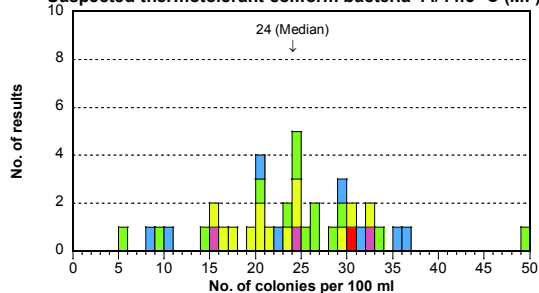
The Swedish standard states incubation at 44 °C but one laboratory reported 44.5 °C. The temperature 44 °C is also stated in EN ISO 9308-1:2000. The three laboratories using Norwegian standard has incubated at 44.5 °C, according to the standard, while

Standard, Method	Tot n	A					B					C				
		n	Med	CV	F	< >	n	Med	CV	F	< >	n	Med	CV	F	< >
Total	39	39	24	—	—	—	39	100	—	—	—	38	0	—	1	—
EN ISO 9308-1	8	8	26	—	—	—	8	142	—	—	—	7	0	—	1	—
SS 028167	14	14	25	—	—	—	14	112	—	—	—	14	0	—	0	—
SFS 4088	13	13	21	—	—	—	13	100	—	—	—	13	0	—	0	—
NS 4792	3	3	24	—	—	—	3	87	—	—	—	3	0	—	0	—
Other/Unknown	1	1	30	—	—	—	1	220	—	—	—	1	0	—	0	—

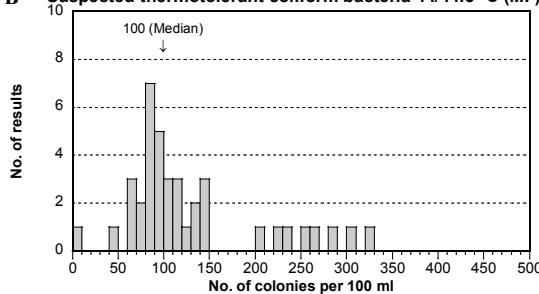
A Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



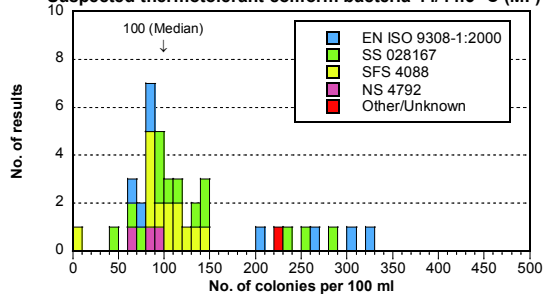
Suspected thermotolerant coliform bacteria 44/44.5 °C (MF)



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



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



all laboratories using Finnish standard has incubated at 44 °C, also according to that standard.

No general correlation between standard, and thus indirectly temperature, and colony recovery can be seen. The median value, as well as the mean value (not shown), is highest with Lactose TTC Agar according to EN ISO 9308-1:2000 in mixture B due to a tail with high results.

Mixture A

- Two strains of *E. coli* and *K. pneumoniae* appear with various nuances of blue colonies on m-FC at 44/44.5 °C. The corresponding colonies are orange to yellow on LTTC.

Mixture B

- The strain of *E. coli* grows with blue colonies on m-FC at 44/44.5 °C. The colonies are orange to yellow on LTTC. *Citrobacter freundii* may appear with small blue or yellow colonies, respectively, when the temperature is a bit below 44 °C. Normally they will not grow.
- The tail with high results probably is caused by some colonies of *C. freundii* appearing. There is reason to question if the temperature has been above 43.5 °C in those cases.

Mixture C

- No thermotolerant coliform bacteria were included in the mixture but one false positive result was recorded.

***Escherichia coli* (MF)**

E. coli is quantified after confirmation of colonies that have grown either at 36±2 °C or at 44/44.5 °C. The primary growth media LTTC or LES are used at 36 °C and LTTC or m-FC at 44/44.5 °C. The results from the two temperatures are here shown in separate tables. The 9-10 results with unclear incubation temperature are not separately shown but are included only in the table "All results".

There was one strain of *E. coli* present in each of mixture A and B. Neither at 36±2 nor at 44/44.5 °C was a general difference between methods seen, probably partly due to the very few results from several media. There is not even a pattern seen for e.g. Lactose TTC Agar compared to other media between the two temperatures.

All results

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	76	70	13	20	4	0	2	74	113	23	1	–	–	76	0	–	0	–	–

Primary incubation at 36±2 °C

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	54	51	12	20	2	0	1	53	113	22	1	0	0	54	0	–	0	–	–
m-Endo Agar LES	41	40	12	19	1	0	0	40	116	22	1	0	0	41	0	–	0	–	–
Lactose TTC Agar	8	7	9	27	1	0	0	8	106	15	0	0	0	8	0	–	0	–	–
Chromocult C. Agar	3	2	15	–	0	0	1	3	119	–	0	0	0	3	0	–	0	–	–
Other/Unknown	2	2	15	–	0	0	0	2	62	–	0	0	0	2	0	–	0	–	–

Primary incubation at 44/44.5 °C

Medium/Standard	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	12	9	14	24	2	0	1	12	112	30	0	0	0	12	0	–	0	–	–
<u>Medium</u>																			
m-FC Agar	6	5	15	21	1	0	0	6	88	25	0	0	0	6	0	–	0	–	–
Lactose TTC Agar	5	4	12	–	1	0	0	5	126	36	0	0	0	5	0	–	0	–	–
Other/Unknown	1	0	6	–	–	–	–	1	200	–	0	0	0	1	0	–	0	–	–
<u>Standard</u>																			
EN ISO 9308-1	6	4	12	–	1	0	1	6	137	32	0	0	0	6	0	–	0	–	–
SS 028167	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
SFS 4088	1	1	19	–	0	0	0	1	145	–	0	0	0	1	0	–	0	–	–
NS 4792	3	2	14	–	1	0	0	3	78	–	0	0	0	3	0	–	0	–	–
Other/Unknown	2	2	14	–	0	0	0	2	79	–	0	0	0	2	0	–	0	–	–

Mixture A

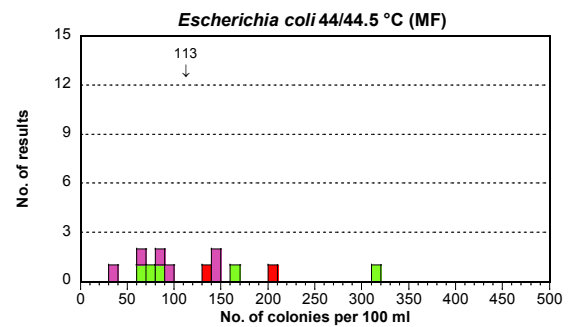
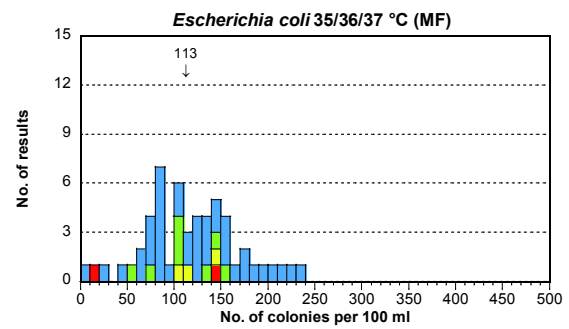
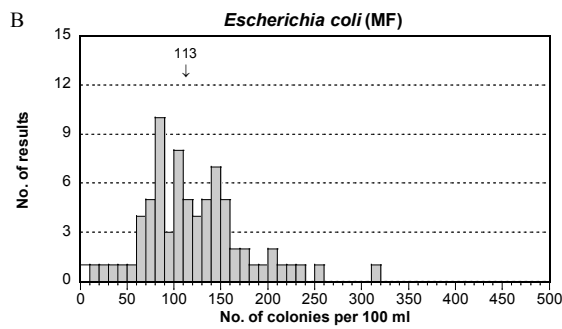
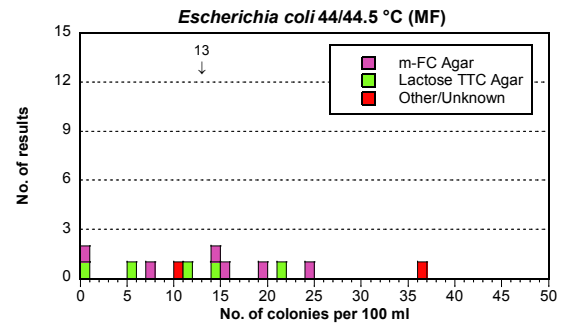
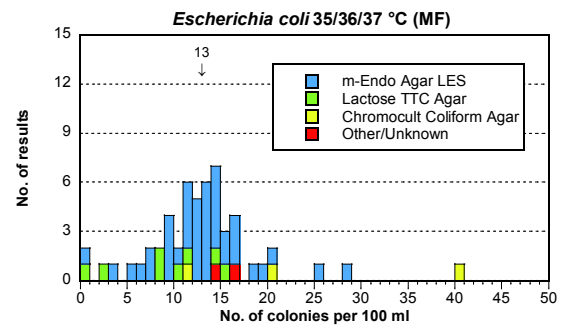
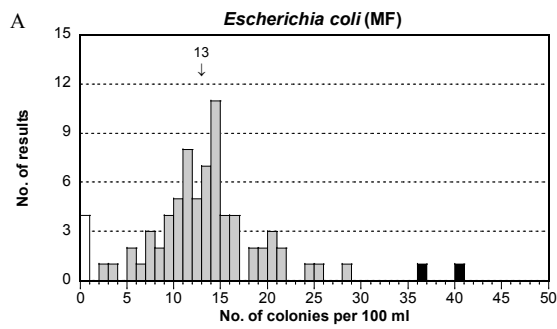
- One typical *E. coli* strains was included in the mixture. Also *K. pneumoniae* appear as presumptive *E. coli*, both at 36±2 and 44/44.5 °C. To confirm the presence of *E. coli*, test of indole production or β -glucuronidase activity has to be used.
- The result distribution was good but with a tendency to be over-represented by low results. An indication is the four false negative results. For one of these also the result for coliform bacteria is zero. For the rest there is no clear explanation.

Mixture B

- Another strain of *E. coli* than in mixture A was included here. *C. freundii* that will appear at least at 36±2 will be removed as presumptive *E. coli* by confirmation due to the lack of indole production and β -glucuronidase activity.
- The result distribution was relatively good. One false negative result was found.

Mixture C

- No *E. coli* was included in the mixture and no false positive result was recorded.



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

The rapid method used for both these parameters was almost exclusively Colilert® Quanti-Tray® from the manufacturer IDEXX Inc. with incubation at 35, 36 or 37 °C. One laboratory has not used a rapid method but the classical multiple tube method with MPN quantification (Standard Methods 9221B; 5) for coliform bacteria. 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. One laboratory in the category Other/Unknown stated the use of "Colilert 24 hours".

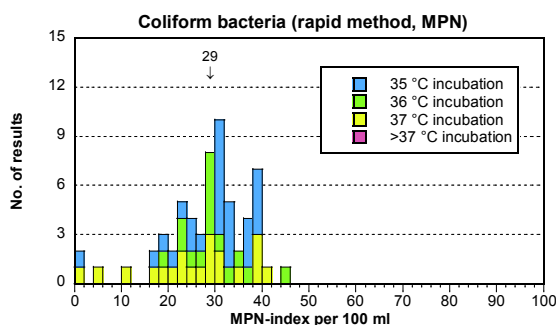
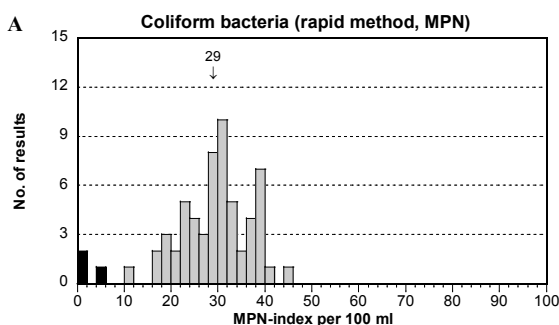
For coliform bacteria there was a tendency in all mixtures in the previous round that the trays with 51 wells gave somewhat lower average recovery than trays with 97 wells. This could not be seen this time. As many as 14 outlying results were present, but only from 5 laboratories with 2 or 4 outliers each. Eight (2×4) of those results was the outcome when the common logarithm was used instead of ordinary numbers. One laboratory with 2 results probably has forgotten to give the results for the proper

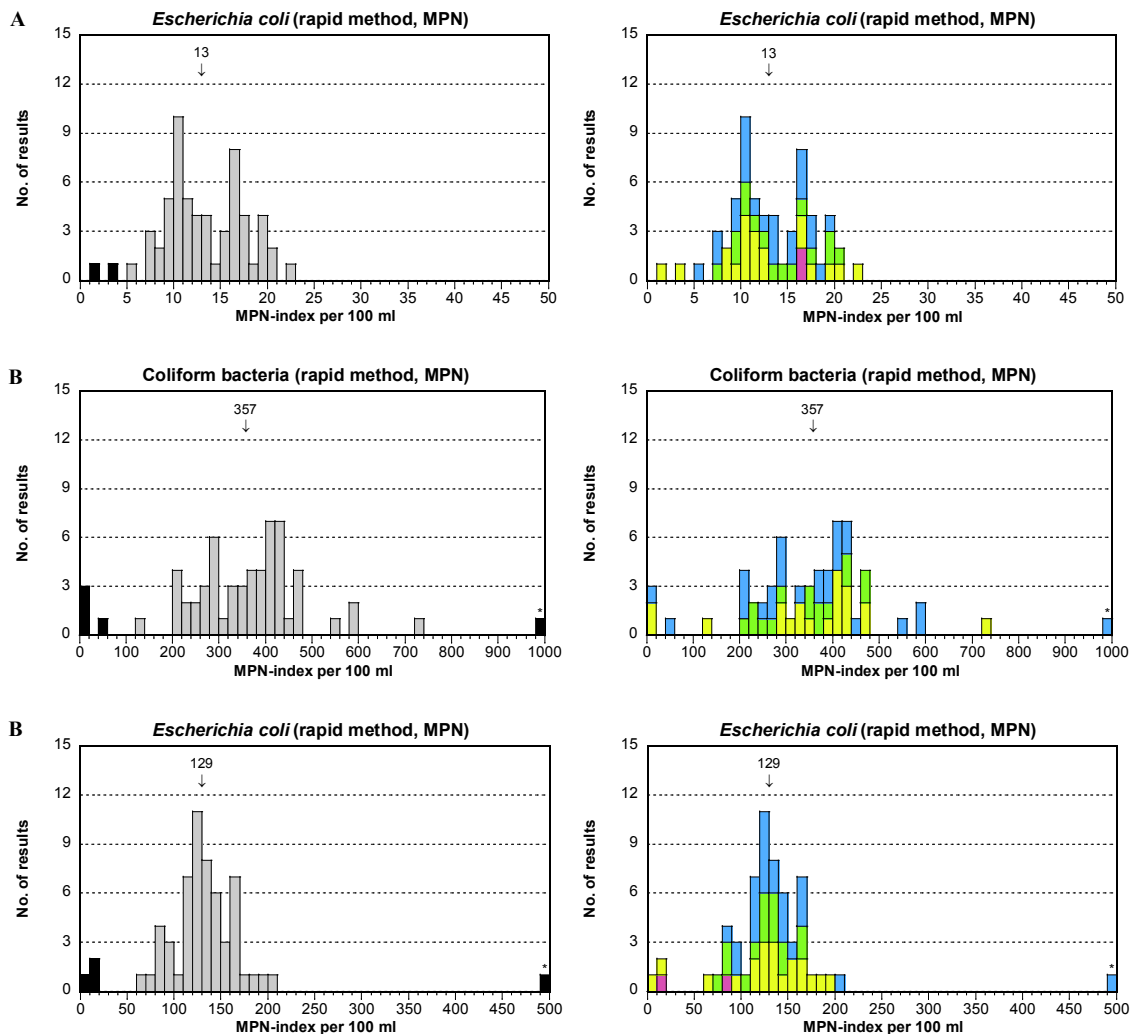
Coliform bacteria, Rapid method with MPN

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	60	57	29	13	0	3	0	56	357	15	0	3	1	60	0	–	0	–	–
Colilert Quanti-51	15	14	31	10	0	1	0	14	364	18	0	1	0	15	0	–	0	–	–
Colilert Quanti-97	42	41	28	13	0	1	0	40	352	14	0	1	1	42	0	–	0	–	–
Colilert Quanti-?	1	1	26	–	0	0	0	1	435	–	0	0	0	1	0	–	0	–	–
Other/Unknown	2	1	39	–	0	1	0	1	360	–	0	1	0	2	0	–	0	–	–
Not rapid method	1	1	23	–	0	0	0	0	–	–	0	1	0	1	0	–	0	–	–

E. coli, Rapid method with MPN

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, Rapid meth.	59	57	13	16	0	2	0	56	129	12	0	2	1	59	0	–	0	–	–
Colilert Quanti-51	16	15	13	15	0	1	0	14	140	14	0	1	1	16	0	–	0	–	–
Colilert Quanti-97	41	40	12	16	0	1	0	40	125	11	0	1	0	41	0	–	0	–	–
Colilert Quanti-?	1	1	17	–	0	0	0	1	138	–	0	0	0	1	0	–	0	–	–
Other/Unknown	1	1	16	–	0	0	0	1	120	–	0	0	0	1	0	–	0	–	–
Not rapid method	1	1	16	–	0	0	0	0	0	–	0	1	0	1	0	–	0	–	–





volume after using a tenfold dilution. For the 4 remaining results there is no clear reason.

Mixture A

- Both the strain of *E. coli* and *K. pneumoniae* will grow in the medium and possess the enzyme β -galactosidase. They are thus detected as coliform bacteria by methods based on the activity of this enzyme (ONPG positive), e.g. Colilert[®]-18/24 Quanti-Tray[®] where ONPG is a substrate (giving yellow colour).
- The enzyme β -glucuronidase (fluorescence) detects the *E. coli* strain only.
- There seems to be two peaks for *E. coli* results, but without a clear reason.

Mixture B/C

- *C. freundii* and *E. coli* were detected as coliform bacteria and *E. coli*, respectively, in a corresponding way as the coliform bacteria in mixture A.

Mixture C

- No *E. coli* was included in the mixture and no false positive result was recorded.

Presumptive and confirmed *Clostridium perfringens* (MF)

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

There is one method explicitly described in the Drinking water directive (4), the use of m-CP Agar incubated at 44 °C. The method includes a confirmation step with ammonia vapour, where a red coloration of colonies indicates *C. perfringens*. Due to the hesitation in many countries to use this method, the use of a standard still under process (ISO/CD 6461-2:2002-12-20, CD = Committee Draft) was accepted by the responsible group under the EU Commission. Adjustments in the draft approved in ISO meetings have been pointed out in the instructions for the proficiency testing rounds. The ISO version of the standard is available since November 2013. It has obtained the designation ISO 14189 (2013) and is basically equivalent to the CD version from 2002 after adjustments but has a much more simplified confirmation step. In the new standard, isolated colonies are only tested for activity of the enzyme acid phosphatase. When the new standard has been included in directives and regulations, the CD version will be invalid to use. The standard is under the process of being accepted also as an EN standard, probably in 2015, and will thus become a national standard in most European countries.

Only one participant has used a different method, that is NMKL 95:5 (2009), modified. That result is included in the group Other/Unknown.

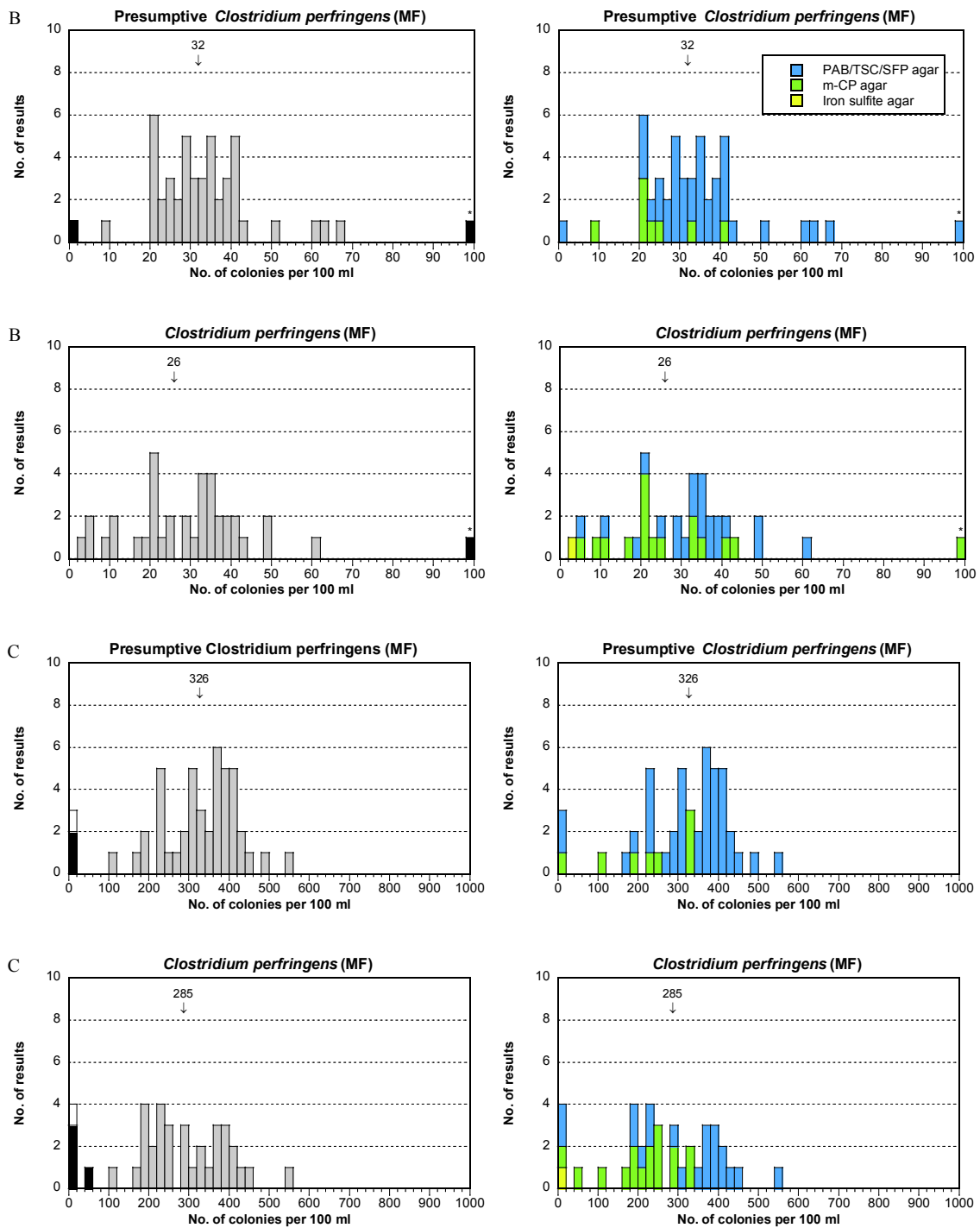
Using m-CP Agar yielded lower numbers for both presumptive *C. perfringens* and *C. perfringens* in both mixture B and C than using PAB/TSC/SFP Agar. The histograms show clearly that the results of m-CP Agar are mainly in the lower end of the results. The dispersion, CV, was in three of four cases the largest with m-CP agar. All these results may not be valid for all strains but at least for the strain of *C. perfringens* used in the test items.

Presumptive *Clostridium perfringens* MF

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	47	42	0	–	5	–	–	45	32	17	0	1	1	44	326	15	1	2	0
PAB/TSC/SFP agar	39	34	0	–	5	–	–	37	34	15	0	1	1	37	343	13	1	1	0
m-CP agar	8	8	0	–	0	–	–	8	23	21	0	0	0	11	242	18	0	1	0
Other/Unknown	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–

Clostridium perfringens MF

Medium	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	38	37	0	–	1	–	–	37	26	27	0	0	1	33	285	17	1	4	0
PAB/TSC/SFP agar	21	20	0	–	1	–	–	21	31	22	0	0	0	19	332	15	1	1	0
m-CP agar	16	16	0	–	0	–	–	15	22	26	0	0	1	14	227	14	0	2	0
Other/Unknown	1	1	0	–	0	–	–	1	2	–	0	0	0	0	–	–	0	1	0



Mixture A

- No presumptive *C. perfringens* was included in the mixture. Yet, there were 5 false positive results for the presumptive analysis and one for *C. perfringens*.

Mixtures B and C

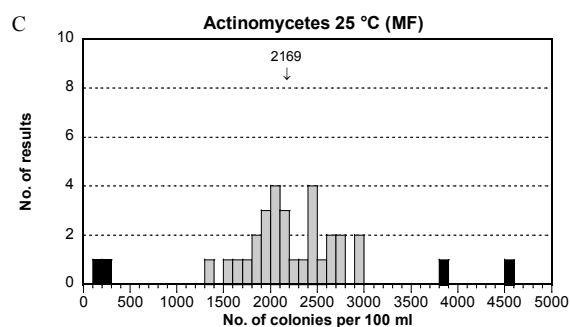
- The same strain of *C. perfringens* was present in both mixtures.
- The colour of the colonies with PAB/TSC/SFP may vary from light grey-brown to completely black, at least partly depending on the condition of the medium and its reduction potential.
- The distribution of the results was less scattered than often during previous rounds, which is seen both from the histograms and the CV. Only for *C. perfringens* in mixture B the somewhat larger dispersion made it difficult to identify outliers.
- Deviating results, in particular low ones, are present among all methods used and for both analyses.

Actinomycetes (MF)

The analysis of actinomycetes is included because it is among the methods that should regularly be used 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). Modifications of the method are not accepted and because of that, no method data was asked for. Hence, no grouping according to methods is done.

All results

Medium	Tot n	A					B					C				
		n	M _v	CV	F	< >	n	M _v	CV	F	< >	n	M _v	CV	F	< >
Total	33	33	0	–	0	– –	32	0	–	1	– –	33	2169	10	0	2 2



Mixtures A and B

- There was no actinomycete in the mixture. One false positive result was recorded in mixture B.

Mixture C

- One actinomycete within the group *Streptomyces sp.* was included. Two low and two high outliers were present, but generally the laboratories quantified the bacterium correctly. The dispersion was small.

Moulds and yeasts (MF)

Out of the 42 laboratories that analysed moulds and yeasts, 33 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. Two Finnish laboratories used Glucose Yeast Extract agar with oxytetracycline as inhibiting substance (OGYE) according to the standard ISO 7954 (1987) and two other Finnish laboratories used DRBC (see below) connected to NMKL 98, modified. Four Finnish laboratories used "Malt Extract Agar" (MEA), out of which two in conjunction with SFS 5507 and the other without mentioning any standard. Sabouraud agar was used by one Swedish laboratory. The last to media mentioned is included in the group Other/Unknown.

Various names, some appropriate and other probably inappropriate, were reported for the media linked to the use of SS 028192. These are "Cooke Rose Bengal" agar, "Rose Bengal agar base", "Rose Bengal Chloramphenicol" (RBC) agar and "Dichloran Rose Bengal Chloramphenicol" (DRBC) agar. According to the standard, dichloran should not be an ingredient (and thus DRBC should not be used) but instead Rose Bengal and the two stronger inhibitory substances chlortetracycline and chloramphenicol are authorized. Both of them are usually used by the Swedish laboratories. That medium is here designated RBCC agar from the English spelling of the ingredients. Another name used is "Rose Bengal agar according to Burman", based on its promoter. Many laboratories report the use of only one of the substances, which is usually in accordance with the recommendations from the manufacturer of the respective medium. Many laboratories using a commercial medium in which Rose Bengal is included have not reported the use of Rose Bengal. What is base medium and supplements compared to finished medium is not clear. The information reported for the media and inhibitors are thus in many cases ambiguous.

Only 4-5 laboratories have used other temperature than 25 °C and other incubation time than 7 days. Therefore, in this round the grouping by methods is according to the media the laboratories have reported. In the group RBC agar all types of RBC agar are included irrespectively of which supplements are used. Among the others it is only DRBC agar "Water" (implying that it is used together with the standard SS 028192 or its national counterparts) that has been used by more than four laboratories. Thus, it is not meaningful to discuss differences in results between other groups.

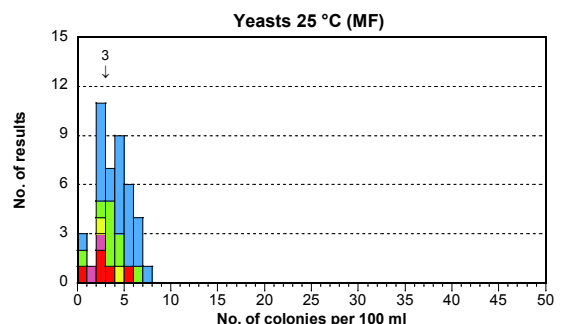
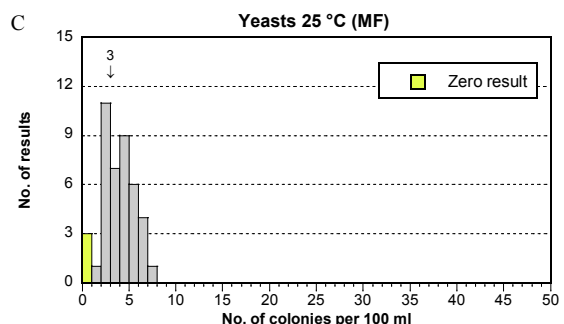
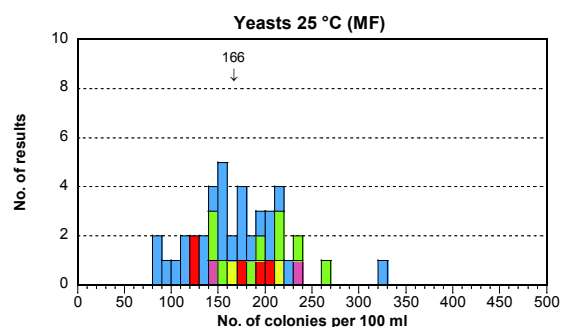
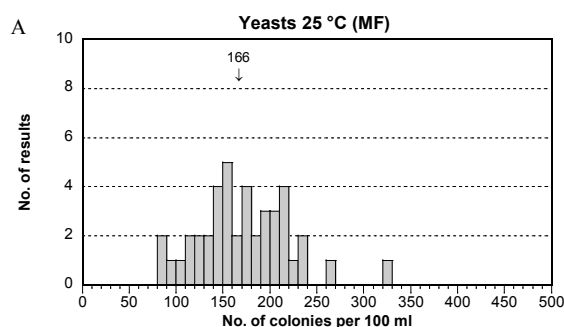
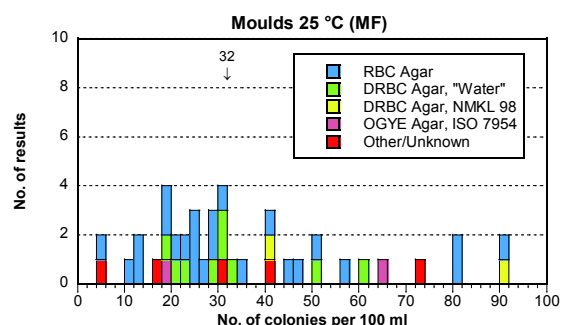
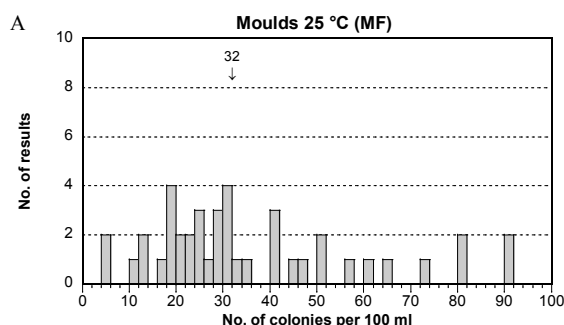
There is a tendency that the results are somewhat higher with DRBC agar "Water" compared to RBC agar for yeasts in mixture A. Also other media used have higher averages than RBC agar for that analysis.

Moulds MF

Standard, Method	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	42	29	15	13	10	0	2	32	87	39	2	0	7	40	0	–	1	–	–
RBC A(gar)	24	24	31	34	0	0	0	23	0	–	1	–	–	24	0	–	0	–	–
DRBC A "Water"	9	9	31	20	0	0	0	2	0	–	0	–	–	9	0	–	0	–	–
DRBC A NMKL 98	2	2	63	–	0	0	0	2	0	–	0	–	–	2	0	–	0	–	–
OGYE A ISO 7954	2	2	39	–	0	0	0	2	0	–	0	–	–	2	0	–	0	–	–
Other/Unknown	5	5	28	45	0	0	0	5	0	–	0	–	–	5	0	–	0	–	–

Yeasts MF

Standard, Method	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total	42	31	0	–	9	–	–	34	482	12	7	0	0	38	837	6	1	1	2
RBC A(gar)	24	24	156	16	0	0	0	23	0	–	1	–	–	24	4	30	0	0	0
DRBC A "Water"	9	9	189	11	0	0	0	9	0	–	0	–	–	9	3	41	0	0	0
DRBC A NMKL 98	2	2	184	–	0	0	0	2	0	–	0	–	–	2	3	–	0	0	0
OGYE A ISO 7954	2	2	182	–	0	0	0	2	0	–	0	–	–	2	1	–	0	0	0
Other/Unknown	5	5	158	12	0	0	0	4	0	–	1	–	–	5	2	61	0	0	0



Mixture A

- The mould *Phialophora malorum* and the yeast *Kluyveromyces maraxianus* were present in the mixture.
- The distribution of mould results are scattered and the dispersion large, which made it difficult to identify outliers. The yeasts were more abundant than the moulds and could possibly grow and conceal small or undeveloped mould colonies. This might be one explanation for the scattered results. The tail with high results for moulds seems to contradict this explanation. However, there is a possibility that the majority of laboratories with lower results have underestimated the number of mould colonies just because some of them were difficult to see. If that is true, there has been more accuracy in counting by the laboratories with high results for moulds.
- In spite of a better distribution for the yeasts, there were no deviating results.

Mixture B

- The mixture contained no yeasts or moulds. No false positive results were present.

Mixture C

- Two moulds *Hanseniaspora malorum* and *Kluyveromyces maraxianus* were present in the mixture. The concentrations were very low for both of them, often so low that *K. maraxianus* didn't appear at all.
- The distribution of the results was good, despite the low content. The 3 zero results are not reckoned as wrong but acceptable when the average is as low as 3 cfu/100 ml.

Culturable microorganisms 22 °C, 3 days

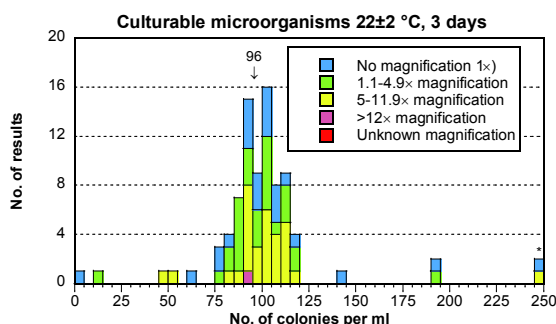
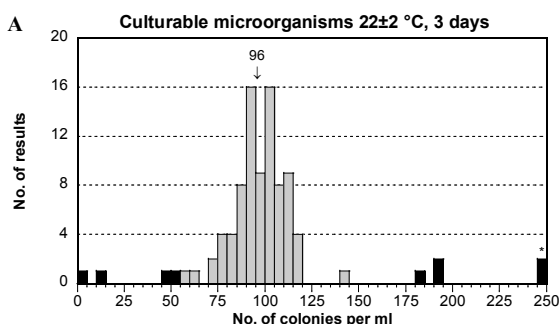
Eighty six of the 93 participating laboratories reported the use of EN ISO 6222:1999. Seven laboratories used Plate Count Agar (PCA), of which 5 together with EN ISO 6222:1999. PCA was otherwise used in combination with NMKL 86, 1999 or "Standard methods" (5). One laboratory used Nutrient Agar with membrane filtration and "Nutrient pads" but otherwise unknown method. Three more laboratories reported other current or previous national methods.

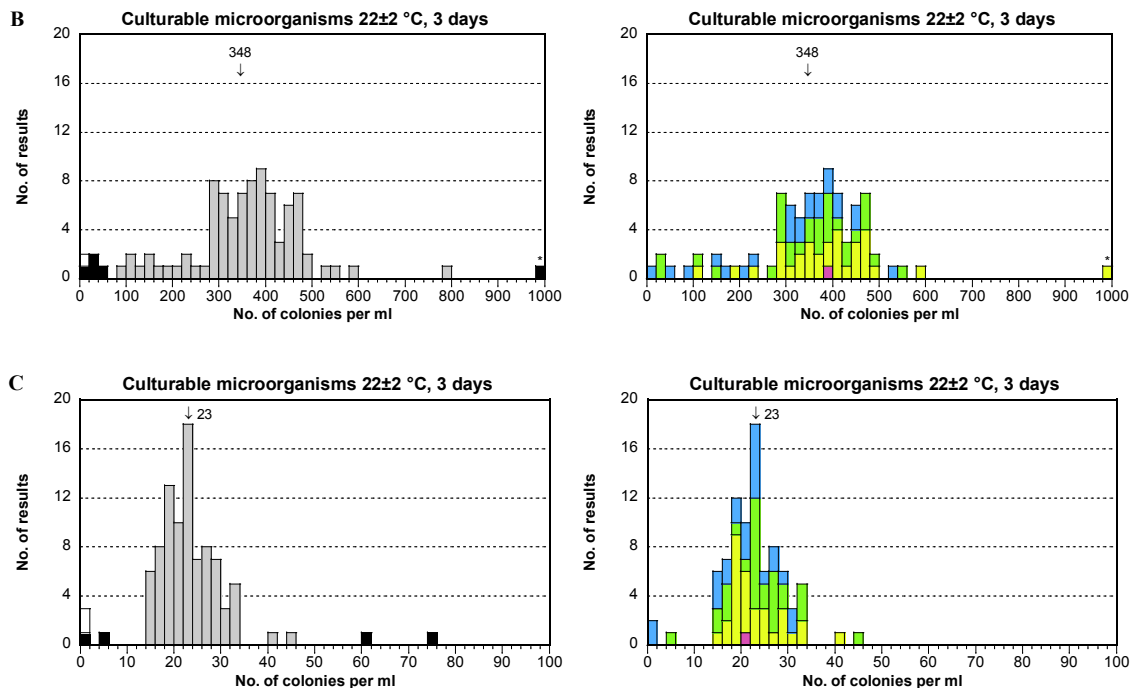
Only comparisons of method variants in connection to EN ISO 6222:1999 are shown and discussed. Results are grouped for culture media and magnification for reading.

Plate Count Agar has a lower average in mixture B, but only 5 laboratories used that medium. There is a tendency to lower recovery with no and low magnification (<5×) than with higher magnification, at least in mixture B. In the tail of 14 low results in that mixture, based on EN ISO 6222:1999, magnification >4,9× have been used by only 3 laboratories. The reason to the low results are probably small colonies, see below concerning mixture B.

22±2 °C, 3 days

Group of results	Tot n	A						B						C					
		n	Mv	CV	F	<	>	n	Mv	CV	F	<	>	n	Mv	CV	F	<	>
Total, all results	93	92	96	7	0	4	5	92	348	16	1	4	1	93	23	12	2	2	2
EN ISO 6222	86	77	98	6	0	4	4	80	350	15	0	4	1	83	23	12	1	2	0
<u>Medium</u>																			
Yeast extract Agar	81	72	98	6	0	4	4	75	354	15	0	4	1	78	23	12	1	2	0
Plate Count Agar	5	5	98	2	0	0	0	5	286	21	0	0	0	5	23	9	0	0	0
Other/Unknown	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
<u>Magnification</u>																			
None	24	21	97	8	0	1	2	21	315	18	0	2	0	22	21	11	1	1	0
1,1–4,9×	30	27	96	5	0	1	1	28	355	14	0	2	0	29	24	13	0	1	0
5–11,9×	31	28	100	5	0	2	1	30	368	14	0	0	1	31	23	12	0	0	0
> 12×	1	1	94	–	0	0	0	1	390	–	0	0	0	1	21	–	0	0	0
Unknown	0	0	–	–	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–
Other method	7	6	75	–	0	0	1	6	330	30	1	0	0	4	22	11	1	0	2





Mixture A

- Although at least the two coliform bacteria strains are able to appear after 3 days, it is mainly colonies of *Staphylococcus xylosus* that is detected.
- Except the large number of outliers, the distribution was well gathered with a very small dispersion. Four low and 5 high outliers were present.

Mixture B

- The colonies consist almost entirely of *Pseudomonas putida*. Also the two coliform bacteria will grow and appear after 3 days but they are in much lower numbers.
- There is a tail with low results. Beside those values the distribution is good. The colonies of *P. putida* are relatively small after 3 days making it difficult to discern all of them when low magnification was used. In cases with small colonies, the results may be dependent on the eyesight of the person reading the plates, since good eyesight and accuracy is then important. Five low and one high deviating result were identified in the outlier test. In practice, probably more of the low results should be considered as deviating.

Mixture C

- In practice it is only colonies of the actinomycete *Streptomyces sp.* that contribute to the results. In the specific analysis of actinomycetes on a selective medium, the average was 21 cfu/ml and here 23 cfu/ml. This difference is minute but generally it is reasonable with a somewhat higher recovery on an unselective medium than on a selective one.
- The distribution of the results was good and with small dispersion. Four low and 2 high deviating results were recorded.

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 to summarize its performance. These values are highlighted with bold text and colour background in annex A, where all reported results are compiled. The limit values for lowest and highest accepted results are given for each analyse in the summarizing lines at the end of annex A, together with the measurement uncertainty of the mean.

Base for assessment of the performance

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

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

Mixed up results and other practical errors

When whole samples seem to have been mixed up, the corresponding sample numbers are hatched in annex A. In this round no laboratory seems to have mixed up individual results or vials. Two laboratories have erroneously reported results with common logarithms, leading to several low outliers. Furthermore, a number of laboratories have several deviating results that are somewhat dependent. Some laboratories seem to have forgotten to report the results for the volume asked for.

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

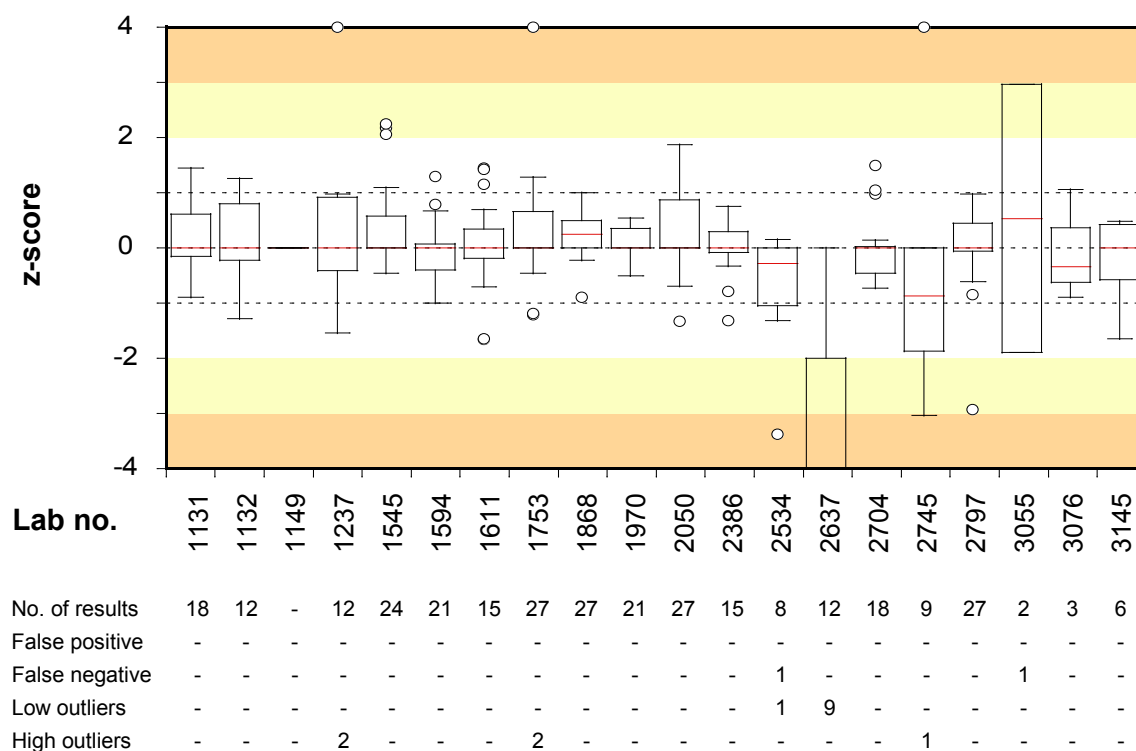
The square root transformed results of the laboratories are calculated to standard scores, z-scores, to be comparable between analyses. They are shown in annex B but not further evaluated. They are given explicitly to facilitate the follow-up process for the laboratories using z-scores in control charts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

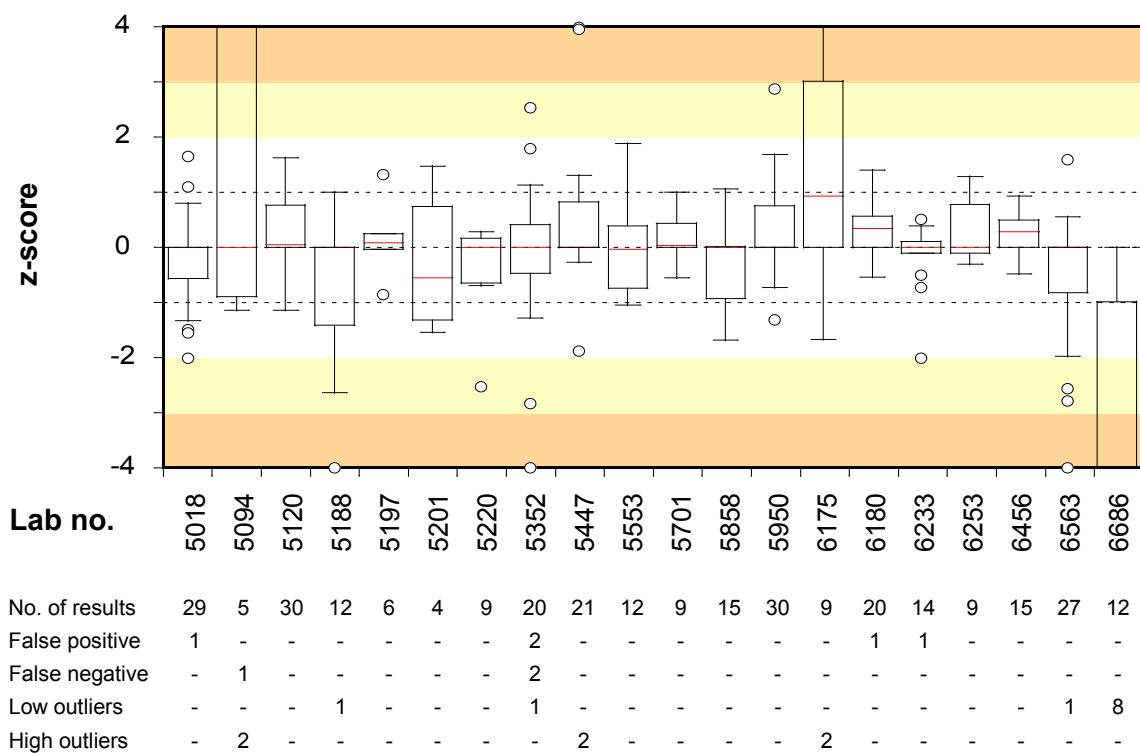
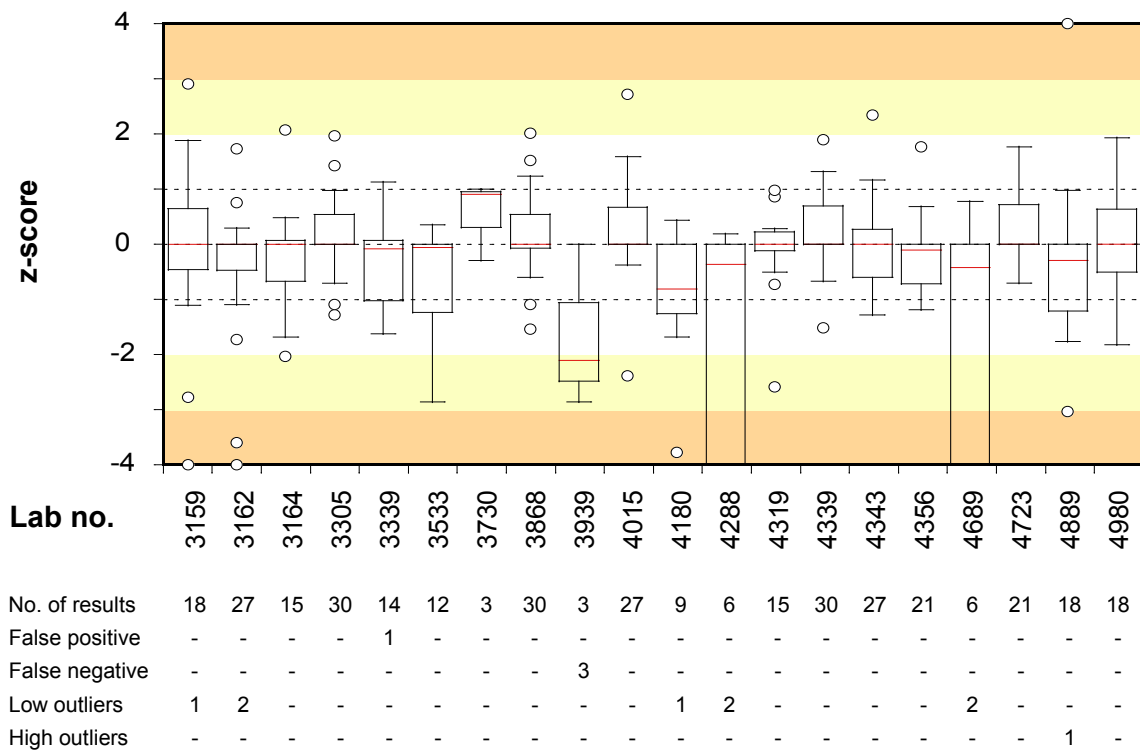
The z-scores are the base for the box plots. The range of the z-scores for each laboratory is shown by a rectangle (box) and lines and/or circles above and beneath the box. The smaller the range from lowest to highest value is in the plot and the more centred around zero the values are, the better is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

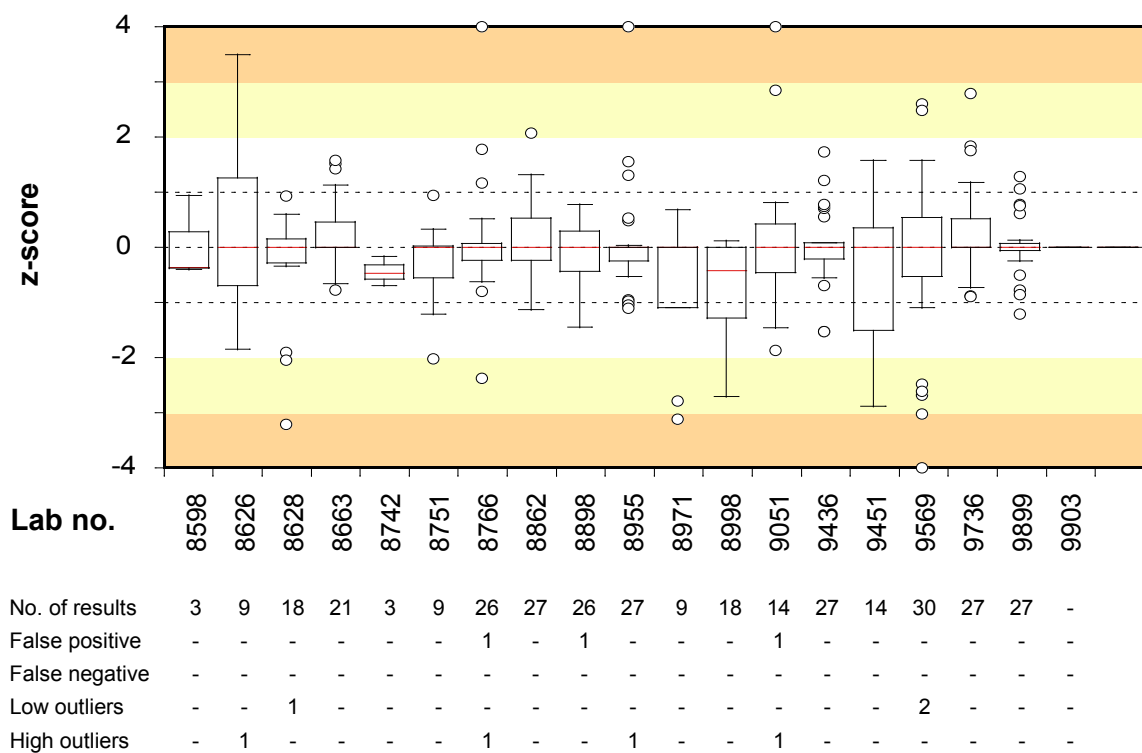
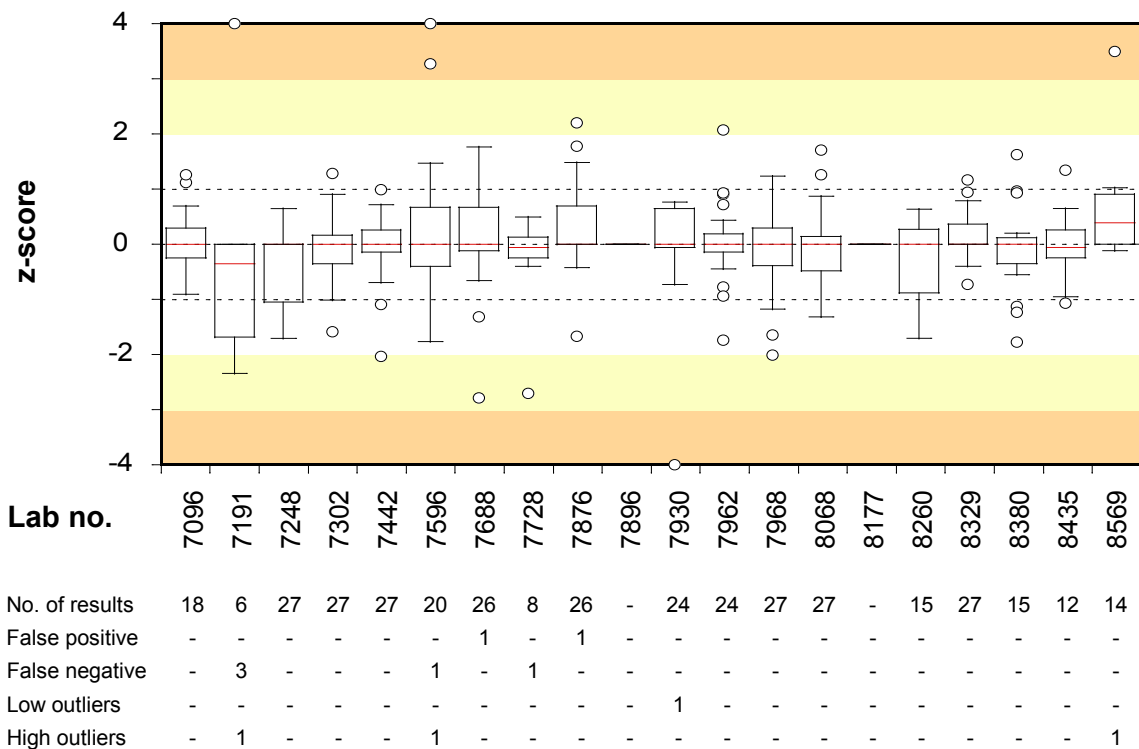
Box plots and numbers of deviating results for each participating laboratory

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

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







Test material, quality controls and processing of data

Description of the test material

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

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

Table 2 *Microorganisms present in the mixtures*

Mixture ¹	Microorganisms	Strain no.	cfu/100 ml ²
A	<i>Escherichia coli</i>	SLV-165	13
	<i>Klebsiella pneumoniae</i>	SLV-537	17
	<i>Phialophora malorum</i>	SLV-545	30
	<i>Kluyveromyces maraxianus</i>	SLV-439	200
	<i>Staphylococcus xylosus</i>	SLV-283	99 *
B	<i>Escherichia coli</i>	SLV-082	120
	<i>Citrobacter freundii</i>	SLV-424	230
	<i>Clostridium perfringens</i>	SLV-442	43
	<i>Pseudomonas putida</i>	SLV-231	560 *
C	<i>Clostridium perfringens</i>	SLV-442	350
	<i>Hanseniaspora uvarum</i>	SLV-555	4
	<i>Kluyveromyces maraxianus</i>	SLV-439	<1
	<i>Streptomyces sp.</i>	SLV-548	2400

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

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 is normally checked by weighing about 10 vials of each mixture. The largest differences between vials in mixture A was 3 mg. The highest accepted difference is

15 mg (3%). In mixtures B and C, where the dispensing was made by a technical device, the calibration of volume was performed through weighing a number of vials before and during the process.

Table 3 presents the results from the organizer in the form of concentration means (cfu) and coefficients of variation (CV) from duplicate analyses of 10 vials from each mixture. The results relate to the volume that was used for counting the colonies. According to the criteria for the CVs used they were acceptable for the mixtures to be considered homogenous. The highest accepted CV is normally 25%. For very low colony counts a higher CV is accepted.

Table 3 Contents (cfu) and measures of homogeneity (CV; coefficient of variation in per cent) in relevant sample volumes for the various parameters in the mixtures ¹

Analysis parameter <i>Method standard for analysis</i>	Mixture					
	A ²		B		C	
	cfu	CV	cfu	CV	cfu	CV
Coliform bacteria (MF) <i>m-Endo Agar LES according to SS 028167</i>	30	5	35	5 ^a	–	–
Suspected thermotolerant colif. bact. (MF) <i>m-FC Agar, 44 °C according to SS 028167</i>	28	8	11	8 ^a	–	–
<i>Escherichia coli</i> (MF) <i>m-Endo Agar LES according to SS 028167</i>	13	7	12	10 ^a	–	–
Presumptive <i>Clostridium perfringens</i> (MF) <i>TSC Agar according to ISO/CD 6461-2:2002</i>	–	–	43	7	35	6 ^a
Moulds (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	5	20 ^a	–	–	–	–
Yeasts (MF) <i>Rose Bengal Agar with both chloramphenicol and chlortetracycline according to SS 028192</i>	15	8 ^a	–	–	9	14 ^b
Actinomycetes (MF) <i>Actinomycete Isolation Agar with cycloheximide according to SS 028212</i>	–	–	–	–	48	6 ^c
Culturable microorg., 3d 22 °C (pour plate) <i>Yeast extract Agar according to SS-EN ISO 6222:1999</i>	100	5	562	3	52	4 ^c

1 n=10 vials analysed in duplicate, normally 100 ml for MF and 1 ml for pour plate, 110, 7, and 6 weeks ahead of the testing round start for the mixtures A, B and C, respectively

2 A stability check of 5 vials with duplicate analyses were performed 6 weeks ahead of the start, showing that the concentrations were still acceptable. The results of that check are used in table 2.

a Result for 10 ml

b Result for 200 ml

c Result for 2 ml

– No target organism and, thus, no analyses

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 transformation of the results since 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.

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

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

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

References

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

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

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2 3 1	26	340	0	26	340	0	-	-	-	10	100	0	35	411	0	16	148	0
1132	2 1 3	-	-	-	-	-	-	15	90	0	7	90	0	28	352	0	16	119	0
1149	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	3 1 2	-	-	-	33	186	0	-	-	-	15	108	0	-	-	-	-	-	-
1545	2 3 1	24	340	0	24	255	0	24	255	0	13	255	0	-	-	-	-	-	-
1594	2 1 3	22	365	0	22	365	0	15	114	0	9	130	0	22	365	0	13	124	0
1611	3 1 2	27	440	0	27	440	0	29	116	0	19	200	0	18	435	0	7	121	0
1753	2 1 3	31	400	0	31	400	0	-	-	-	13	155	0	39	1437	0	17	846	0
1868	1 3 2	30	357	0	30	357	0	-	-	-	13	135	0	36	414	0	16	137	0
1970	2 1 3	28	340	0	28	340	0	29	260	0	14	140	0	-	-	-	-	-	-
2050	1 3 2	-	-	-	29	350	0	-	-	-	12	80	0	37	586	0	18	126	0
2386	3 1 2	22	300	0	22	300	0	-	-	-	11	140	0	-	-	-	-	-	-
2534	1 3 2	28	233	0	28	56	0	-	-	-	12	0	0	-	-	-	-	-	-
2637	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	1.51	2.84	<1,00	1.04	2.69	<1,00
2704	1 3 2	-	-	-	24	300	0	-	-	-	10	170	0	29	478	<1	10	178	<1
2745	3 2 1	16	210	0	16	210	0	5	60	0	5	60	0	-	-	-	-	-	-
2797	3 1 2	20	290	0	11	290	0	20	300	0	11	160	0	23	411	0	12	111	0
3055	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3145	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	32	411	0	7	112	0
3159	1 3 2	-	-	-	26	30	0	-	-	-	16	15	0	23.8	738	<1	8.7	192	<1
3162	2 1 3	24	250	0	24	250	0	-	-	-	13	84	0	31	249	0	11	82	0
3164	3 1 2	-	-	-	28	230	<1	-	-	-	25	75	<1	31	411	<1	10	128	<1
3305	1 2 3	23	320	0	23	320	0	-	-	-	7	160	0	45	360	0	19	160	0
3339	2 3 1	26	360	0	26	360	0	-	-	-	8	130	0	-	-	-	-	-	-
3533	1 2 3	-	-	-	27	80	0	-	-	-	14	80	0	-	-	-	-	-	-
3730	3 2 1	22	310	0	-	-	-	33	118	0	-	-	-	-	-	-	-	-	-
3868	1 3 2	28	250	0	28	250	0	20	80	0	14	110	0	41	340	0	22	120	0
3939	1 2 3	10	80	<	<	80	<	10	80	<	-	-	-	-	-	-	-	-	-
4015	2 3 1	34	600	0	34	600	0	83	139	0	14	150	0	31	548	0	5	138	0
4180	3 1 2	-	-	-	30	180	0	-	-	-	10	75	0	-	-	-	-	-	-
4288	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	1	1	<1	-	-	-
4319	1 3 2	33	310	0	33	310	0	24	100	0	3	89	0	28	380	0	10	160	0
4339	2 1 3	33	292	0	33	292	0	23	124	0	20	234	0	26	435	0	17	138	0
4343	3 2 1	30	297	0	30	297	0	-	-	-	12	81	0	38	291	0	17	93	0
4356	2 3 1	40	210	0	40	210	0	21	98	0	16	60	0	34	248	0	10	108	0
4689	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	23	49	0	16	13	0
4723	1 3 2	32	300	0	32	300	0	-	-	-	11	131	0	-	-	-	-	-	-
4889	3 2 1	-	-	-	24	190	0	-	-	-	2	57	0	19	330	0	12	95	0
4980	3 2 1	32	140	0	32	140	0	24	60	0	24	60	0	30.6	429	<1	16.4	165.2	<1
5018	1 3 2	18	315	0	18	158	0	-	-	-	11	158	0	16	299	0	8	125	0
5094	1 2 3	9300	290	0	-	-	-	8	62	0	0	62	0	-	-	-	-	-	-
5120	2 1 3	35	410	0	35	410	0	32	130	0	13	140	0	38	410	0	20	130	0
5188	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5197	3 2 1	-	-	-	-	-	-	-	-	-	20	112	0	-	-	-	-	-	-
5201	2 1 3	21	-	0	-	-	-	-	-	-	21	-	0	-	-	-	-	-	-
5220	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	30.9	290.9	0	13.4	125.9	0
5352	1 3 2	35	480	0	35	480	0	14	140	0	14	140	0	-	-	-	-	-	-
5447	2 1 3	-	-	-	60	340	0	-	-	-	40	100	0	-	-	-	-	-	-
5553	2 3 1	-	-	-	22	200	<1,0	-	-	-	18	80	<1,0	-	-	-	-	-	-
5701	3 1 2	30	290	0	30	290	0	30	220	0	10	130	0	-	-	-	-	-	-
5858	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	29	200	<1	9	83	<1
5950	1 3 2	29	400	0	34	400	0	29	90	0	11	98	0	20	436.2	0	10	185	0
6175	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	36	200	0	15	200	0
6180	1 2 3	30	340	0	30	340	0	30	145	0	18	111	0	25	340	0	15	145	0
6233	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	29	413	0	10	132	0
6253	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	39	360	0	16	120	0
6456	2 3 1	-	-	-	29	270	0	-	-	-	14	90	0	33	460	0	14	148	0
6563	2 1 3	24	281	<1	24	281	<1	24	281	<1	6	211	<1	30	262	<1	15	145	<1
6686	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	5.37	19.08	0	3.7	11.39	0
7096	1 2 3	-	-	-	29	250	0	-	-	-	14	101	0	31	324	0	11	165	0
7191	1 3 2	110	280	100	0	0	0	-	-	-	0	80	0	-	-	-	-	-	-
7248	2 1 3	22	223	0	22	200	0	25	42	0	16	42	0	24	219	0	9	92	0
7302	3 1 2	23	370	0	23	370	0	26	93	0	11	190	0	23	461	0	9	135	0
7442	1 3 2	24	300	0	24	300	0	-	-	-	14	171	0	24	383	0	12	146	0
7596	3 1 2	90	320	0	90	320	0	32	87	0	0	87	0	38	305	0	17	122	0
7688	4 2 3	-	-	-	39	330	0	-	-	-	14	120	0	28	290	0	19	150	0
7728	3 1 2	-	-	-	12	335	0	-	-	-	0	108	0	-	-	-	-	-	-
7876	1 3 2	26	380	0	26	150	0	20	105	0	11	150	0	33	445	0	19	152	0
7896	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean					27	286	0				13	113	0	29	357	0	13	129	0
CV (%)					12	17	-				20	23	-	13	15	-	16	12	-

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

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

Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0	50	370	-	-	-	-	-	-	-	-	-	-	-	-	110	320	18	1131
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	108	450	30	1132
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1149
-	-	-	0	210	220	-	-	-	-	-	-	-	-	-	190	470	15	1237
0	39	550	0	39	550	30	0	0	146	0	3	0	0	2300	96	415	29	1545
-	-	-	-	-	-	65	0	0	140	0	2	-	-	-	91	430	24	1594
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	87	340	22	1611
0	20	223	-	-	-	28	0	0	156	0	6	0	0	1986	103	382	22	1753
0	37	373	-	-	-	28	0	0	217	0	4	0	0	2273	108	472	18	1868
0	33	280	0	33	280	33	0	0	190	0	4	-	-	-	94	390	21	1970
0	43	421	-	-	-	40	0	0	175	0	5	0	0	1655	119	471	25	2050
0	33	330	0	33	330	-	-	-	-	-	-	-	-	-	94	440	16	2386
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	86	298	16	2534
<1,00	1.57	2.64	-	-	-	-	-	-	-	-	-	-	-	-	2	2.68	1.41	2637
-	-	-	0	17	280	-	-	-	-	-	-	-	-	-	96	350	19	2704
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	350	87	19	2745
0	34	390	0	34	390	30	0	0	140	0	4	-	-	-	102	403	28	2797
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	140	165	0	3055
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	111	310	18	3076
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3145
-	-	-	0	20	280	-	-	-	-	-	-	-	-	-	117	454	26	3159
0	27	310	-	-	-	18	0	0	157	0	3	0	0	220	54	440	33	3162
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	154	20	3164
0	40	360	0	40	360	30	0	0	190	0	2	0	0	2400	87	330	17	3305
12	20	190	0	20	190	-	-	-	-	-	-	-	-	-	91	490	23	3339
-	-	-	0	11	180	-	-	-	-	-	-	-	-	-	101	206	22	3533
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	110	460	21	3730
0	41	230	0	41	230	60	0	0	230	0	6	0	0	2400	98	340	15	3868
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	70	<	<	3939
0	40	486	-	-	-	25	0	0	151	0	5	0	0	2100	94	491	21	4015
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	50	17	4180
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93	370	20	4288
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	360	22	4319
0	31	430	0	31	430	20	0	0	210	0	2	0	0	2000	77	362	23	4339
0	63	414	-	-	-	18	0	0	117	0	5	0	0	1829	109	289	19	4343
0	28	230	0	28	230	-	-	-	-	-	-	-	-	-	87	303	22	4356
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4689
0	35	291	-	-	-	57	0	0	134	0	2	0	0	2636	114	580	27	4723
-	-	-	0	42	250	-	-	-	-	-	-	-	-	-	190	310	14	4889
-	-	-	0	32	250	-	-	-	-	-	-	-	-	-	83	293	14	4980
0	32	370	0	32	370	16	0	0	121	5200	2	0	0	2900	101	270	29	5018
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	440	1300	18	5094
0	34	370	0	34	370	13	0	0	150	0	2	0	0	2700	97	520	27	5120
-	-	-	0	2	10	19	0	0	150	0	3	-	-	-	110	150	27	5188
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85	367	24	5197
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	82	-	15	5201
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	119	19	5220
24	66	0	24	11	0	12	0	0	182	0	2	0	0	198	62	300	29	5352
-	-	-	0	48	400	5	0	0	170	0	5	0	0	2600	100	400	22	5447
-	-	-	<1,0	60	192	-	-	-	-	-	-	-	-	-	95	340	27	5553
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	110	420	20	5701
<1	21	250	<1	21	250	-	-	-	-	-	-	-	-	-	111	471	27	5858
0	38	380	0	38	380	27	0	0	220	0	4	0	0	1960	102	468	41	5950
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	180	780	60	6175
3	35	440	0	35	440	-	-	-	-	-	-	-	-	-	99	478	25	6180
-	-	-	-	-	-	4	0	0	170	300	2	-	-	-	101	395	22	6233
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	110	330	22	6253
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	109	400	21	6456
<1	35	364	0	5.48	15.81	10	<1	<1	85	<1	<1	<1	<1	2018	48	116	19	6563
-	-	-	0	37	203	-	-	-	-	-	-	-	-	-	10.34	20.74	4.8	6686
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	93	370	30	7096
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	132	75	7191
0	39	263	-	-	-	45	0	0	116	0	4	0	0	1730	90	236	15	7248
0	26	355	-	-	-	25	0	0	99	0	5	0	0	1800	98	355	25	7302
0	31	391	-	-	-	20	0	0	83	0	4	0	0	2036	106	432	17	7442
0	22	180	0	22	180	-	-	-	-	-	-	-	-	-	117	390	44	7596
-	-	-	0	19	380	23	0	0	260	0	0	0	350	2700	93	540	16	7688
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91	380	22	7728
2	61	395	-	-	-	80	0	0	200	0	7	0	0	2000	100	370	21	7876
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7896
0	32	326	0	26	285	32	0	0	166	0	3	0	0	2169	96	348	23	Mean
-	17	15	-	27	17	32	-	-	14	-	36	-	-	10	7	16	12	CV (%)

Lab no.	Sample	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria ("rapid" MPN)			<i>E. coli</i> ("rapid" MPN)		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7930	1 3 2	32	360	0	32	360	0	-	-	-	16	100	0	25	384	0	10	111	0
7962	1 2 3	32	310	0	32	310	0	16	87	0	9	70	0	26	461	0	11	133	0
7968	1 3 2	33	330	0	33	330	0	17	89	0	19	145	0	16	330	0	7	117	0
8068	2 3 1	24	280	0	24	280	0	19	84	0	9	220	0	20	260	0	9	110	0
8177	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8260	1 3 2	20	149	<1	20	149	<1	31	79	<1	14	79	<1	-	-	-	-	-	-
8329	3 2 1	27	339	0	27	339	0	-	-	-	15	120	0	26	387	0	10	148	0
8380	1 2 3	20	385	0	20	385	0	-	-	-	10	105	0	36	237	0	20	81	0
8435	1 3 2	-	-	-	26	270	0	-	-	-	11	150	0	-	-	-	-	-	-
8569	3 2 1	31	391	0	31	391	0	28	142	0	12	141	0	-	-	-	-	-	-
8598	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8626	2 1 3	36	200	0	36	200	0	36	200	0	36	200	0	-	-	-	-	-	-
8628	1 3 2	-	-	-	28	260	<1	-	-	-	15	36	<1	-	-	-	-	-	-
8663	2 1 3	38	350	0	38	350	0	24	100	0	15	180	0	30	290	0	19	120	0
8742	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8751	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	29	238	<1	11	75	<1
8766	1 3 2	24	300	<1	24	240	<1	23	230	<1	12	120	<1	30	365	<1	13	122	<1
8862	1 2 3	20	380	0	20	380	0	-	-	-	20	118	0	39	420	0	9	150	0
8898	3 1 2	29	345	0	29	345	0	-	-	-	14	118	0	31	287	0	16	89	0
8955	1 3 2	-	-	-	21	270	0	-	-	-	8	140	0	28	260	0	12	130	0
8971	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8998	1 2 3	12	200	0	12	200	0	-	-	-	7	120	0	19.7	218.7	<1	10.7	114.5	<1
9051	2 3 1	8600	409	4100	8600	368	4100	22	327	3900	5	310	0	-	-	-	-	-	-
9436	2 3 1	26	400	0	26	160	0	26	94	0	13	80	0	33	437	0	13	168	0
9451	3 1 2	29	320	0	29	320	0	35	84	0	21	84	0	-	-	-	-	-	-
9569	1 2 3	47	350	<1	47	350	<1	20	8	<1	28	21	<1	11	127	<1	11	62	<1
9736	3 1 2	28	373	0	28	373	0	-	-	-	13	109	0	32	582	0	10	167	0
9899	2 1 3	26	282	0	26	282	0	9	73	0	9	73	0	39	426	0	16	163	0
9903	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n	59	58	59	73	73	73	39	39	39	76	75	76	61	61	61	60	60	60
Min	10	80	0	0	0	0	5	8	0	0	0	0	1	1	0	1.04	2.69	0
Max	9300	600	4100	8600	600	4100	83	327	3900	40	310	0	45	1437	0	22	846	0
Median	28	320	0	28	300	0	24	100	0	13	111.5	0	30	365	0	12	129	0
Mean				27	286	0				13	113	0	29	357	0	13	129	0
CV (%)				12	17	-				20	23	-	13	15	-	16	12	-
False positive				0	0	1				0	0	0	0	0	0	0	0	0
False negative				2	1	0				4	1	0	0	0	0	0	0	0
Outliers, low				0	2	0				0	0	0	3	4	0	2	3	0
Outliers, high				3	0	0				2	0	0	0	1	0	0	1	0
Low limit OK	10	80	0	11	80	0	5	8	0	2	15	0	11	127	0	5	62	0
High limit OK	9300	600	4100	47	600	0	83	327	3900	28	310	0	45	738	0	22	200	0

mv ($\sqrt{\text{Mean}}$)			5.194	16.911	0.000				3.546	10.653	0.000		5.369	18.891	0.000		3.566	11.354	0.000
s ($\text{CV} \cdot \text{mv} / 100$)			0.640	2.794	0.000				0.702	2.444	0.000		0.679	2.848	0.000		0.558	1.331	0.000
$u_{rel,mv}$ (%) ($100 \cdot s / \sqrt{n_{mv}}$)			1.5	2.0					2.4	2.7			1.7	2.0			2.1	1.6	
x ($\sqrt{\text{Result}}$)																			
z ($(x - mv) / s$)																			

Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0	40	390	-	-	-	30	0	0	200	0	4	-	-	-	101	39	27	7930
-	-	-	-	-	-	90	0	0	160	0	4	0	0	1510	109	330	23	7962
0	28	410	0	28	410	19	0	0	230	0	1	-	-	-	88	299	23	7968
0	24	320	0	24	320	40	0	0	210	0	2	-	-	-	100	450	30	8068
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8177
<1	36	234	<1	36	234	-	-	-	-	-	-	-	-	-	100	180	24	8260
0	28	305	-	-	-	35	0	0	170	0	6	0	0	2180	104	445	28	8329
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	99	352	22	8380
-	-	-	0	34	200	-	-	-	-	-	-	-	-	-	115	236	22	8435
0	40	418	-	-	-	-	-	-	-	-	-	0	-	3850	110	381	23	8569
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	91	308	28	8598
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	73	292	19	8626
-	-	-	<1	5	57	28	<1	<1	180	<1	3	-	-	-	109	311	26	8628
0	24	300	0	24	300	-	-	-	-	-	-	-	-	-	97	360	32	8663
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	330	19	8742
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	89	387	28	8751
16	110	310	-	-	-	80	<1	<1	130	<1	6	<1	<1	1300	88	410	21	8766
0	25	354	-	-	-	90	0	0	190	0	5	0	0	1900	90	283	18	8862
0	31	227	-	-	-	24	64	0	145	0	4	0	0	2482	90	397	17	8898
-	-	-	0	48	340	73	0	0	120	0	3	0	0	4500	82	290	21	8955
-	-	-	-	-	-	40	0	0	200	0	0	-	-	-	59	250	17	8971
0	29	161	-	-	-	-	-	-	-	-	-	-	-	-	84	309	23	8998
0	20	320	0	20	160	-	-	-	-	-	-	-	-	-	102	387	25	9051
0	29	400	-	-	-	22	0	0	101	0	2	0	0	2500	92	355	33	9436
0	9	110	0	9	110	-	-	-	-	-	-	-	-	-	-	400	32	9451
<1	21	1	<1	21	1	50	<1	<1	210	<1	3	<1	<1	2400	105	290	32	9569
0	23	418	-	-	-	46	0	0	325	0	3	0	0	2955	100	400	18	9736
0	20	318	-	-	-	50	0	0	169	0	2	0	0	2189	93	364	23	9899
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9903

47	47	47	38	38	38	42	42	42	42	42	42	33	32	33	92	92	93	n
0	1.57	0	0	2	0	4	0	0	83	0	0	0	0	198	2	0	0	Min
24	110	550	24	210	550	90	64	0	325	5200	7	0	350	4500	440	1300	75	Max
0	32	354.5	0	31	280	29	0	0	169.5	0	3	0	0	2180	98	363	22	Median
0	32	326	0	26	285	32	0	0	166	0	3	0	0	2169	96	348	23	Mean
-	17	15	-	27	17	32	-	-	14	-	36	-	-	10	7	16	12	CV (%)
5	0	0	1	0	0	0	1	0	0	2	0	0	1	0	0	0	0	False pos.
0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	False neg.
0	1	2	0	0	4	0	0	0	0	0	0	0	0	2	4	4	2	Outliers <
0	1	0	0	1	0	0	0	0	0	0	0	0	0	2	5	1	2	Outliers >
0	9	110	0	2	110	4	0	0	83	0	0	0	0	1300	59	87	14	Low limit
0	66	550	0	60	550	90	0	0	325	0	7	0	0	2955	140	780	44	High limit

0.000	5.655	18.050	0.000	5.104	16.894	5.691	0.000	0.000	12.872	0.000	1.727	0.000	0.000	46.569	9.808	18.661	4.753	mv
0.000	0.976	2.625	0.000	1.401	2.908	1.834	0.000	0.000	1.846	0.000	0.619	0.000	0.000	4.427	0.683	3.074	0.574	s
	2.6	2.2		4.5	3.0	5.0			2.2		5.5			1.8	0.8	1.8	1.3	u _{rel,mv} (%)
																		x
																		z

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

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			E. coli (MF)			Coliform bacteria ("rapid" MPN)			E. coli ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	2	3	1		-0.149	0.547	0.000				-0.546	-0.267	0.000	0.806	0.486	0.000	0.778	0.610	0.000		
1132	2	1	3		-1.282	-0.477	0.000				-0.114	-0.045	0.000	0.778	-0.335	0.000					
1149	3	2	1																		
1237	3	1	2		0.860	-1.172	0.000				0.466	-0.107	0.000								
1545	2	3	1		-0.461	-0.337	0.000				0.085	2.175	0.000								
1594	2	1	3		-0.787	0.785	0.000				-0.777	0.306	0.000	-0.999	0.075	0.000	0.071	-0.164	0.000		
1611	3	1	2		0.003	1.455	0.000				1.158	1.427	0.000	-1.658	0.690	0.000	-1.649	-0.266	0.000		
1753	2	1	3		0.584	1.106	0.000				0.085	0.735	0.000	1.290	4.000	0.000	0.999	4.000	0.000		
1868	1	3	2		0.442	0.710	0.000				0.085	0.395	0.000	0.930	0.512	0.000	0.778	0.264	0.000		
1970	2	1	3		0.152	0.547	0.000				0.279	0.482	0.000								
2050	1	3	2	0.299	0.643	0.000	-0.116	-0.699	0.000	1.051	1.867	0.000	1.213	-0.097	0.000						
2386	3	1	2	-0.787	0.147	0.000	-0.326	0.482	0.000												
2534	1	3	2	0.152	-3.375	0.000	-0.116		0.000												
2637	3	1	2							-4.000	-4.000	0.000	-4.000	-4.000	0.000						
2704	1	3	2	-0.461	0.147	0.000	-0.546	0.976	0.000	0.024	1.044	0.000	-0.723	1.494	0.000						
2745	3	2	1	-1.866	-0.866	0.000	-1.865	-1.189	0.000												
2797	3	1	2	-2.933	0.042	0.000	-0.326	0.817	0.000	-0.843	0.486	0.000	-0.182	-0.615	0.000						
3055	3	1	2																		
3076	2	1	3																		
3145	2	1	3							0.424	0.486	0.000	-1.649	-0.579	0.000						
3159	1	3	2	-0.149	-4.000	0.000	0.647	-2.774	0.000	-0.722	2.906	0.000	-1.105	1.880	0.000						
3162	2	1	3	-0.461	-0.394	0.000	0.085	-0.609	0.000	0.293	-1.093	0.000	-0.447	-1.727	0.000						
3164	3	1	2	0.152	-0.625	0.000	2.071	-0.815	0.000	0.293	0.486	0.000	-0.723	-0.030	0.000						
3305	1	2	3	-0.622	0.350	0.000	-1.282	0.817	0.000	1.972	0.029	0.000	1.422	0.973	0.000						
3339	2	3	1	-0.149	0.738	0.000	-1.021	0.306	0.000												
3533	1	2	3	0.003	-2.852	0.000	0.279	-0.699	0.000												
3730	3	2	1																		
3868	1	3	2	0.152	-0.394	0.000	0.279	-0.067	0.000	1.523	-0.159	0.000	2.016	-0.300	0.000						
3939	1	2	3		-2.852	0.000															
4015	2	3	1	0.995	2.715	0.000	0.279	0.652	0.000	0.293	1.587	0.000	-2.383	0.296	0.000						
4180	3	1	2	0.442	-1.251	0.000	-0.546	-0.815	0.000												
4288	1	2	3							-4.000	-4.000	0.000									
4319	1	3	2	0.860	0.249	0.000	-2.583	-0.499	0.000	-0.114	0.212	0.000	-0.723	0.973	0.000						
4339	2	1	3	0.860	0.063	0.000	1.320	1.900	0.000	-0.397	0.690	0.000	0.999	0.296	0.000						
4343	3	2	1	0.442	0.115	0.000	-0.116	-0.676	0.000	1.172	-0.643	0.000	0.999	-1.285	0.000						
4356	2	3	1	1.766	-0.866	0.000	0.647	-1.189	0.000	0.681	-1.104	0.000	-0.723	-0.723	0.000						
4689	3	2	1							-0.843	-4.000	0.000	0.778	-4.000	0.000						
4723	1	3	2	0.723	0.147	0.000	-0.326	0.324	0.000												
4889	3	2	1	-0.461	-1.119	0.000	-3.036	-1.269	0.000	-1.487	-0.255	0.000	-0.182	-1.208	0.000						
4980	3	2	1	0.723	-1.818	0.000	1.928	-1.189	0.000	0.240	0.640	0.000	0.867	1.126	0.000						
5018	1	3	2	-1.487	-1.554	0.000	-0.326	0.784	0.000	-2.015	-0.562	0.000	-1.322	-0.131	0.000						
5094	1	2	3					-1.137	0.000												
5120	2	1	3	1.128	1.195	0.000	0.085	0.482	0.000	1.172	0.477	0.000	1.625	0.036	0.000						
5188	3	1	2																		
5197	3	2	1				1.320	-0.029	0.000												
5201	2	1	3				1.477		0.000												
5220	1	3	2							0.280	-0.644	0.000	0.170	-0.100	0.000						
5352	1	3	2	1.128	1.789	0.000	0.279	0.482	0.000												
5447	2	1	3	3.987	0.547	0.000	3.958	-0.267	0.000												
5553	2	3	1	-0.787	-0.991	0.000	0.993	-0.699	0.000												
5701	3	1	2	0.442	0.042	0.000	-0.546	0.306	0.000												
5858	3	1	2							0.024	-1.668	0.000	-1.014	-1.686	0.000						
5950	1	3	2	0.995	1.106	0.000	-0.326	-0.308	0.000	-1.320	0.701	0.000	-0.723	1.689	0.000						
6175	1	3	2							0.930	-1.668	0.000	0.551	2.095	0.000						
6180	1	2	3	0.442	0.547	0.000	0.993	-0.048	0.000	-0.543	-0.159	0.000	0.551	0.517	0.000						
6233	3	2	1							0.024	0.503	0.000	-0.723	0.102	0.000						
6253	3	2	1							1.290	0.029	0.000	0.778	-0.300	0.000						
6456	2	3	1	0.299	-0.172	0.000	0.279	-0.477	0.000	0.553	0.898	0.000	0.315	0.610	0.000						
6563	2	1	3	-0.461	-0.053	0.000	-1.561	1.584	0.000	0.160	-0.950	0.000	0.551	0.517	0.000						
6686	2	3	1							-4.000	-4.000	0.000	-2.944	-4.000	0.000						
7096	1	2	3	0.299	-0.394	0.000	0.279	-0.247	0.000	0.293	-0.313	0.000	-0.447	1.121	0.000						
7191	1	3	2			0.000		-0.699	0.000												
7248	2	1	3	-0.787	-0.991	0.000	0.647	-1.707	0.000	-0.691	-1.437	0.000	-1.014	-1.324	0.000						
7302	3	1	2	-0.622	0.832	0.000	-0.326	1.281	0.000	-0.843	0.906	0.000	-1.014	0.199	0.000						
7442	1	3	2	-0.461	0.147	0.000	0.279	0.992	0.000	-0.691	0.239	0.000	-0.182	0.548	0.000						
7596	3	1	2	4.000	0.350	0.000		-0.542	0.000	1.172	-0.501	0.000	0.999	-0.232	0.000						
7688	1	2	3	1.642	0.449	0.000	0.279	0.123	0.000	-0.114	-0.654	0.000	1.422	0.671	0.000						
7728	3	1	2	-2.703	0.498	0.000		-0.107	0.000												
7876	1	3	2	-0.149	-1.670	0.000	-0.326	0.652	0.000	0.553	0.774	0.000	1.422	0.732	0.000						
7896	2	1	3																		
7930	1	3	2	0.723	0.738	0.000	0.647	-0.267	0.000	-0.543	0.248	0.000	-0.723	-0.615	0.000						
7962	1	2	3	0.723	0.249	0.000	-0.777	-0.935	0.000	-0.397	0.906	0.000	-0.447	0.134	0.000						
7968	1	3	2	0.860	0.449	0.000	1.158	0.568	0.000	-2.015	-0.255	0.000	-1.649	-0.404	0.000						
8068	2	3	1	-0.461	-0.064	0.000	-0.777	1.710	0.000	-1.320	-0.971	0.000	-1.014	-0.651	0.000						
8177	2	3	1																		
8260	1	3	2	-1.128	-1.684	0.000	0.279	-0.722	0.000												
8329	3	2	1	0.003	0.537	0.000	0.466	0.123	0.000	-0.397	0.275	0.000	-0.723	0.610	0.000						
8380	1	2	3	-1.128	0.970	0.000	-0.546	-0.166	0.000	0.930	-1.228	0.000	1.625	-1.769	0.000						
8435	1	3	2	-0.149	-0.172	0.000	-0.326	0.652	0.000												
8569	3	2	1	0																	

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

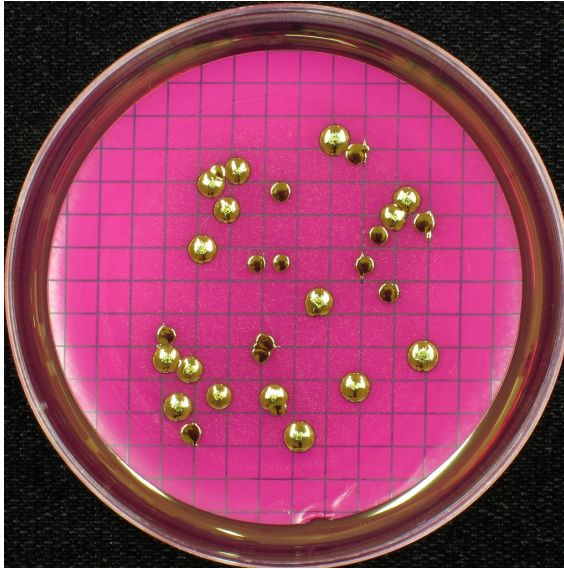
Presumptive C. perfringens (MF)			Clostridium perfringens (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
0.000	1.451	0.452													0.995	-0.251	-0.890	1131
															0.855	0.830	1.262	1132
																		1149
			0.000	4.000	-0.709										4.000	0.982	-1.534	1237
0.000	0.604	2.058	0.000	0.815	2.255	-0.116	0.000	0.000	-0.428	0.000	0.008	0.000	0.000	0.314	-0.015	0.557	1.101	1545
						1.293	0.000	0.000	-0.564	0.000	-0.506				-0.394	0.675	0.254	1594
															-0.704	-0.072	-0.110	1611
0.000	-1.212	-1.187				-0.218	0.000	0.000	-0.207	0.000	1.167	0.000	0.000	-0.453	0.499	0.288	-0.110	1753
0.000	0.438	0.481				-0.218	0.000	0.000	1.007	0.000	0.441	0.000	0.000	0.250	0.855	0.997	-0.890	1868
0.000	0.092	-0.501	0.000	0.457	-0.055	0.029	0.000	0.000	0.494	0.000	0.441				-0.165	0.354	-0.298	1970
0.000	0.924	0.940				0.346	0.000	0.000	0.193	0.000	0.822	0.000	0.000	-1.330	1.611	0.990	0.430	2050
0.000	0.092	0.044	0.000	0.457	0.437										-0.165	0.753	-1.313	2386
															-0.783	-0.455	-1.313	2534
0.000	-4.000	-4.000													-4.000	-4.000	-4.000	2637
			0.000	-0.700	-0.055										-0.015	0.016	-0.688	2704
															4.000	-3.036	-0.688	2745
0.000	0.180	0.647	0.000	0.519	0.982	-0.116	0.000	0.000	-0.564	0.000	0.441				0.426	0.460	0.938	2797
															2.962	-1.892		3055
															1.065	-0.343	-0.890	3076
																		3145
			0.000	-0.451	-0.055										1.476	0.861	0.603	3159
0.000	-0.470	-0.169				-0.790	0.000	0.000	-0.186	0.000	0.008	0.000	0.000	-4.000	-3.600	0.753	1.728	3162
															-1.680	-2.033	-0.490	3164
0.000	0.686	0.352	0.000	0.871	0.715	-0.116	0.000	0.000	0.494	0.000	-0.506	0.000	0.000	0.547	-0.704	-0.161	-1.099	3305
	-1.212	-1.625	0.000	-0.451	-1.070										-0.394	1.131	0.074	3339
			0.000	-1.276	-1.196										0.354	-1.401	-0.110	3533
															0.995	0.907	-0.298	3730
0.000	0.766	-1.098	0.000	0.927	-0.594	1.121	0.000	0.000	1.243	0.000	1.167	0.000	0.000	0.547	0.134	-0.072	-1.534	3868
															-2.110			3939
0.000	0.686	1.522				-0.377	0.000	0.000	-0.317	0.000	0.822	0.000	0.000	-0.168	-0.165	1.138	-0.298	4015
															-1.680	-3.770	-1.099	4180
															-0.241	0.187	-0.490	4288
															0.281	0.102	-0.110	4319
0.000	-0.089	1.023	0.000	0.331	1.322	-0.665	0.000	0.000	0.877	0.000	-0.506	0.000	0.000	-0.417	-1.512	0.119	0.074	4339
0.000	2.338	0.875				-0.790	0.000	0.000	-1.114	0.000	0.822	0.000	0.000	-0.859	0.925	-0.540	-0.688	4343
0.000	-0.372	-1.098	0.000	0.134	-0.594										-0.704	-0.408	-0.110	4356
																		4689
0.000	0.267	-0.377				1.014	0.000	0.000	-0.703	0.000	-0.506	0.000	0.000	1.078	1.272	1.764	0.772	4723
			0.000	0.983	-0.372										4.000	-0.343	-1.763	4889
			0.000	0.395	-0.372										-1.021	-0.502	-1.763	4980
0.000	0.002	0.452	0.000	0.395	0.805	-0.922	0.000	0.000	-1.015		-0.506	0.000	0.000	1.645	0.354	-0.725	1.101	5018
															4.000	4.000	-0.890	5094
0.000	0.180	0.452	0.000	0.519	0.805	-1.137	0.000	0.000	-0.339	0.000	-0.506	0.000	0.000	1.218	0.060	1.348	0.772	5120
			0.000	-2.634	-4.000	-0.726	0.000	0.000	-0.339	0.000	0.008				0.995	-2.086	0.772	5188
															-0.862	0.162	0.254	5197
															-1.102	-1.534		5201
															0.281	-2.522	-0.688	5220
2.529				-1.276		-1.214	0.000	0.000	0.335	0.000	-0.506	0.000	0.000	-4.000	-2.831	-0.436	1.101	5352
			0.000	1.302	1.068	-1.884	0.000	0.000	0.090	0.000	0.822	0.000	0.000	0.999	0.281	0.436	-0.110	5447
			0.000	1.886	-1.045										-0.090	-0.072	0.772	5553
															0.995	0.596	-0.490	5701
0.000	-1.099	-0.853	0.000	-0.372	-0.372										1.065	0.990	0.772	5858
0.000	0.522	0.550	0.000	0.757	0.894	-0.270	0.000	0.000	1.062	0.000	0.441	0.000	0.000	-0.519	0.426	0.967	2.876	5950
															4.000	3.015	4.000	6175
	0.267	1.115	0.000	0.580	1.404	-2.013	0.000	0.000	0.090		-0.506				0.207	1.042	0.430	6180
															0.354	0.395	-0.110	6233
															0.995	-0.161	-0.110	6253
															0.925	0.436	-0.298	6456
0.000	0.267	0.392				-1.379	0.000	0.000	-1.979	0.000	-2.790	0.000	0.000	-0.372	-4.000	-2.567	-0.688	6563
			0.000	-1.972	-4.000										-4.000	-4.000	-4.000	6686
			0.000	0.699	-0.910										-0.241	0.187	1.262	7096
															-1.680	-2.333	4.000	7191
0.000	0.604	-0.698				0.555	0.000	0.000	-1.139	0.000	0.441	0.000	0.000	-1.124	-0.470	-1.073	-1.534	7248
0.000	-0.570	0.302				-0.377	0.000	0.000	-1.584	0.000	0.822	0.000	0.000	-0.936	0.134	0.059	0.430	7302
0.000	-0.089	0.657				-0.665	0.000	0.000	-2.038	0.000	0.441	0.000	0.000	-0.327	0.713	0.691	-1.099	7442
0.000	-0.988	-1.765	0.000	-0.295	-1.196										1.476	0.354	3.277	7596
			0.000	-0.532	0.894	-0.488	0.000	0.000	1.762	0.000	-2.790	0.000		1.218	-0.241	1.489	-1.313	7688
															-0.394	0.271	-0.110	7728
2.208	0.695					1.774	0.000	0.000	0.688	0.000	1.484	0.000	0.000	-0.417	0.281	0.187	-0.298	7876
																		7896
0.000	0.686	0.647				-0.116	0.000	0.000	0.688	0.000	0.441				0.354	-4.000	0.772	7930
						2.070	0.000	0.000	-0.121	0.000	0.441	0.000	0.000	-1.742	0.925	-0.161	0.074	7962
0.000	-0.372	0.838	0.000	0.134	1.154	-0.726	0.000	0.000	1.243	0.000	-1.175				-0.626	-0.445	0.074	7968
0.000	-0.774	-0.061	0.000	-0.146	0.342	0.346	0.000	0.000	0.877	0.000	-0.506				0.281	0.830	1.262	8068
																		8177
0.000	0.353	-1.048	0.000	0.640	-0.549										0.281	-1.706	0.254	8260
0.000	-0.372	-0.223				0.123	0.000	0.000	0.090	0.000	1.167	0.000	0.000	0.028	0.571	0.792	0.938	8329
															0.207	0.033	-0.110	8380
			0.000	0.519	-0.946										1.340	-1.073	-0.110	8435
0.000	0.686	0.912										0.000		3.497	0.995	0.279	0.074	8569
															-0.394	-0.361	0.938	8598
			0.000	-2.047	-3.214	-0.218	0.000	0.000	0.295	0.000	0.008				-1.850	-0.512	-0.688	8626
0.000	-0.774	-0.278	0.000	-0.146	0.147										0.925	-0.334	0.603	8628
															0.060	0.102	1.575	8663
															-0.470	-0.161	-0.688	8742

Lab no.	Sample			Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria ("rapid" MPN)			<i>E. coli</i> ("rapid" MPN)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
8751	3	1	2																		
8766	1	3	2				-0.461	-0.508	0.000				-0.116	0.123	0.000	0.024	-1.216	0.000	-0.447	-2.024	0.000
8862	1	2	3				-1.128	0.924	0.000				1.320	0.086	0.000	0.160	0.075	0.000	0.071	-0.232	0.000
8898	3	1	2				0.299	0.595	0.000				0.279	0.086	0.000	1.290	0.563	0.000	-1.014	0.671	0.000
8955	1	3	2				-0.955	-0.172	0.000				-1.021	0.482	0.000	0.293	-0.685	0.000	0.778	-1.443	0.000
8971	1	3	2													-0.114	-0.971	0.000	-0.182	0.036	0.000
8998	1	2	3				-2.703	-0.991	0.000				-1.282	0.123	0.000	-1.369	-1.441	0.000	-0.528	-0.491	0.000
9051	2	3	1				4.000	0.813					-1.865	2.845	0.000	0.553	0.707	0.000	0.071	1.208	0.000
9436	2	3	1				-0.149	-1.526	0.000				0.085	-0.699	0.000						
9451	3	1	2				0.299	0.350	0.000				1.477	-0.609	0.000	0.424	1.838	0.000	-0.723	1.179	0.000
9569	1	2	3				2.596	0.643	0.000				2.487	-2.483	0.000	-3.021	-2.676	0.000	-0.447	-2.615	0.000
9736	3	1	2				0.152	0.860	0.000				0.085	-0.087	0.000	0.424	1.838	0.000	-0.723	1.179	0.000
9899	2	1	3				-0.149	-0.042	0.000				-0.777	-0.863	0.000	1.290	0.614	0.000	0.778	1.062	0.000
9903	1	3	2																		
n				0	0	0	71	72	72	0	0	0	72	74	76	61	61	61	60	60	60
Min							-2.933	-4.000	0.000				-3.036	-2.774	0.000	-4.000	-4.000	0.000	-4.000	-4.000	0.000
Max							4.000	2.715	0.000				3.958	2.845	0.000	1.972	4.000	0.000	2.016	4.000	0.000
Median							0.152	0.147	0.000				0.085	-0.038	0.000	0.024	0.029	0.000	-0.182	-0.064	0.000
Mean							0.169	-0.102	0.000				0.104	0.000	0.000	-0.197	-0.197	0.000	-0.116	-0.133	0.000
SD							1.270	1.161	0.000				1.163	1.000	0.000	1.308	1.487	0.000	1.171	1.414	0.000
z<-3							0	2	0				1	0	0	4	4	0	1	3	0
-3≤z<-2							3	2	0				1	2	0	2	1	0	2	2	0
-2<z≤3							1	1	0				2	2	0	0	1	0	1	1	0
z>3							3	0	0				2	0	0	0	1	0	0	1	0

Presumptive <i>C. perfringens</i> (MF)			<i>Clostridium perfringens</i> (MF)			Moulds (MF)			Yeasts (MF)			Actinomycetes (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
	4.000	-0.169				1.774	0.000	0.000	-0.797	0.000	1.167	0.000	0.000	-2.375	-0.548	0.329	0.938	8751
0.000	-0.671	0.292				2.070	0.000	0.000	0.494	0.000	0.822	0.000	0.000	-0.673	-0.626	0.517	-0.298	8766
0.000	-0.089	-1.136				-0.432		0.000	-0.450	0.000	0.441	0.000	0.000	0.734	-0.470	-0.598	-0.890	8862
			0.000	1.302	0.531	1.556	0.000	0.000	-1.039	0.000	0.008	0.000	0.000	4.000	-0.470	0.411	-1.099	8898
						0.346	0.000	0.000	0.688	0.000	-2.790				-1.102	-0.531	-0.298	8955
0.000	-0.276	-2.042													-3.113	-0.927	-1.099	8971
0.000	-1.212	-0.061													-0.941	-0.352	0.074	8998
0.000	-0.276	0.743	0.000	-0.451	-1.460	-0.546	0.000	0.000	-1.529	0.000	-0.506	0.000	0.000	0.775	0.426	0.329	0.430	9051
0.000	-2.720	-2.880	0.000	-1.502	-2.203										-0.317	0.059	1.728	9436
0.000	-1.099	-4.000	0.000	-0.372	-4.000	0.753	0.000	0.000	0.877	0.000	0.008	0.000	0.000	0.547		0.436	1.575	9451
0.000	-0.880	0.912				0.595	0.000	0.000	2.793	0.000	0.008	0.000	0.000	1.760	0.642	-0.531	1.575	9569
0.000	-1.212	-0.083				0.753	0.000	0.000	0.069	0.000	-0.506	0.000	0.000	0.049	0.281	0.436	-0.890	9736
															-0.241	0.136	0.074	9899
																		9903
42	47	46	37	38	37	42	41	42	42	40	42	33	31	33	92	91	91	n
0.000	-4.000	-4.000	0.000	-2.634	-4.000	-2.013	0.000	0.000	-2.038	0.000	-2.790	0.000	0.000	-4.000	-4.000	-4.000	-4.000	Min
0.000	4.000	2.058	0.000	4.000	2.255	2.070	0.000	0.000	2.793	0.000	1.484	0.000	0.000	4.000	4.000	4.000	4.000	Max
0.000	0.002	0.168	0.000	0.363	-0.372	-0.167	0.000	0.000	0.080	0.000	0.008	0.000	0.000	0.028	0.134	0.102	-0.110	Median
0.000	0.000	-0.174	0.000	0.105	-0.411	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.015	0.048	-0.129	0.000	Mean
0.000	1.285	1.279	0.000	1.181	1.528	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.660	1.565	1.340	1.291	SD
0	1	2	0	0	4	0	0	0	0	0	0	0	0	2	5	5	2	Sum
0	1	2	0	2	1	1	0	0	1	0	3	0	0	1	2	5	0	36
0	3	1	0	0	1	2	0	0	1	0	0	0	0	0	1	0	1	34
0	1	0	0	1	0	0	0	0	0	0	0	0	0	2	5	2	3	19
																		21

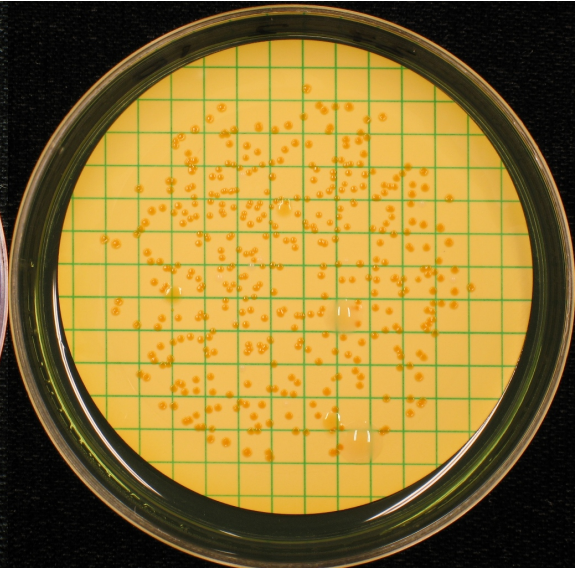
Mixture A

m-Endo Agar LES, 37 °C



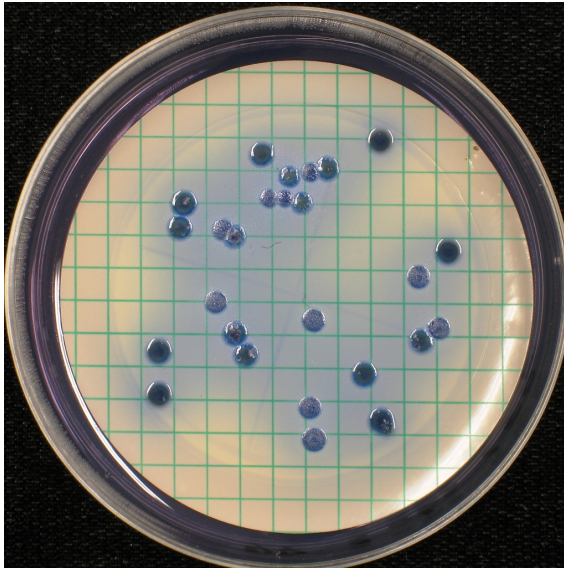
100 ml

m-Lactose TTC Agar, 37 °C



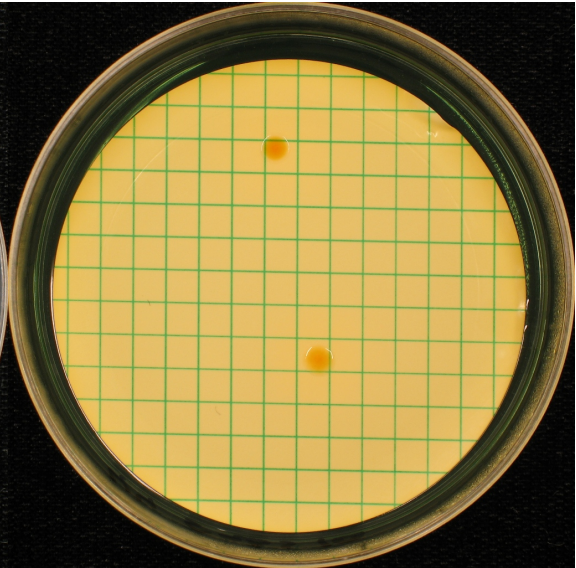
10 ml

m-FC Agar, 44 °C



100 ml

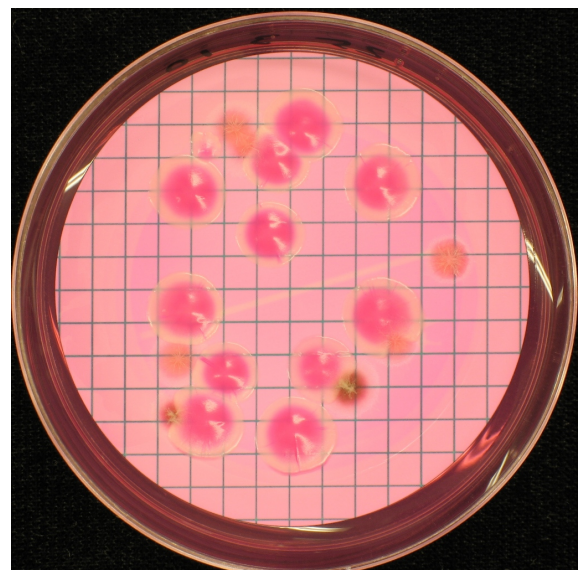
m-Lactose TTC Agar, 44 °C



10 ml

m-TSC Agar, 44 °C

No clostridia present

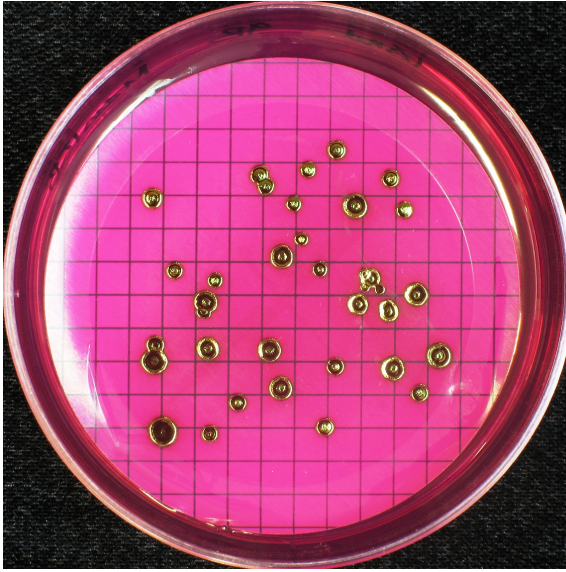


10 ml, 7 days

m-RBCC Agar, 25 °C

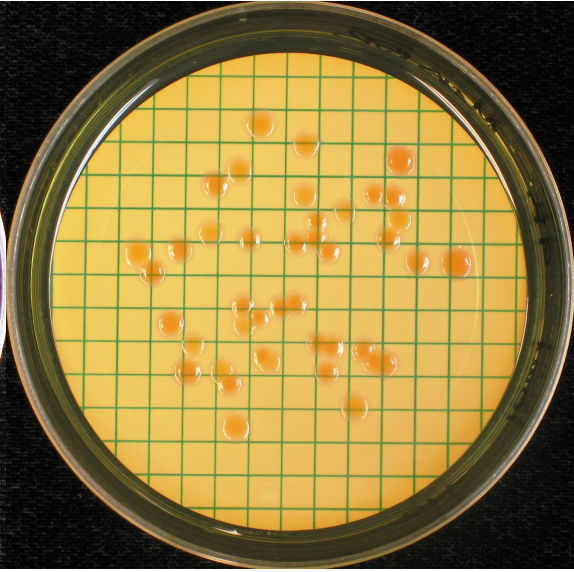
Mixture B

m-Endo Agar LES, 37 °C



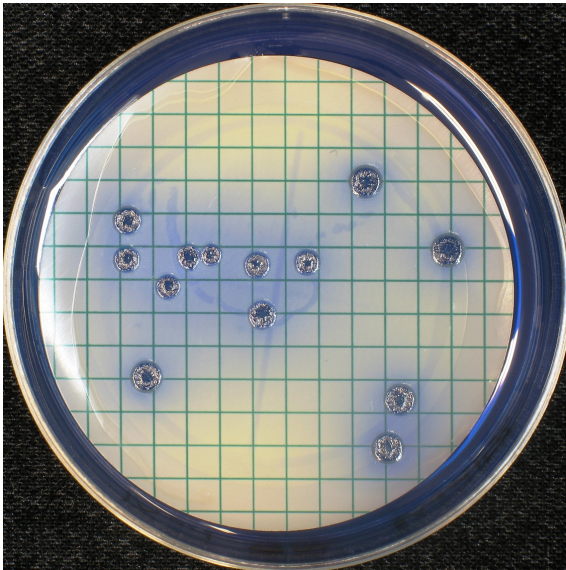
10 ml

m-Lactose TTC Agar, 37 °C



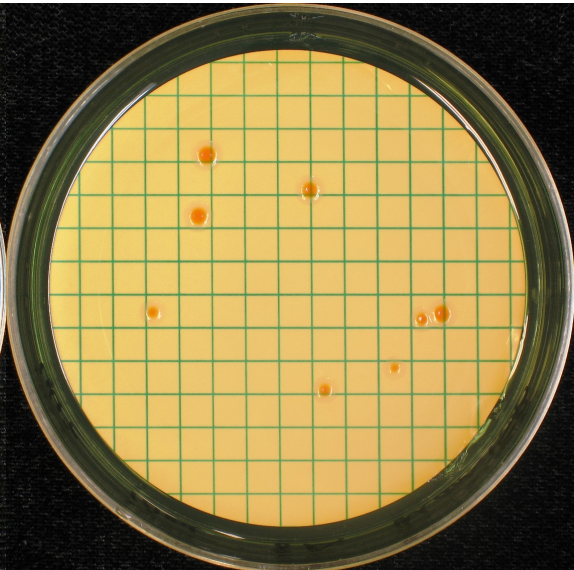
10 ml

m-FC Agar, 44 °C



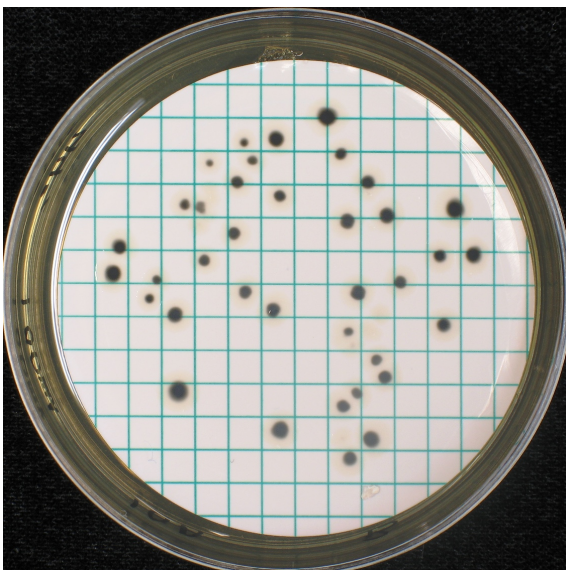
10 ml

m-Lactose TTC Agar, 44 °C



10 ml

m-TSC Agar, 44 °C



100 ml

m-RBCC Agar, 25 °C

No moulds or yeasts present

Mixture C

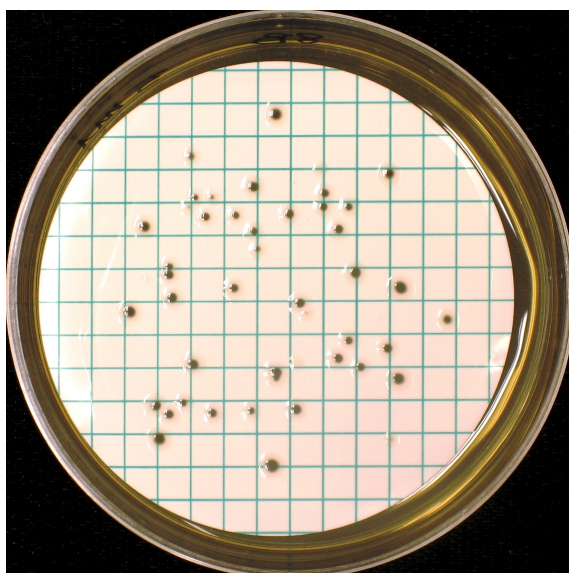
m-Endo Agar LES, 37 °C

No coliforms present

No coliforms present

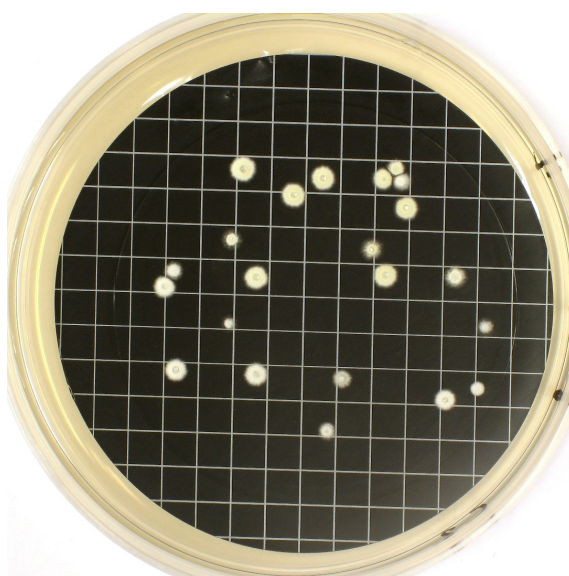
m-Lactose TTC Agar, 37 °C

m-TSC Agar, 44 °C

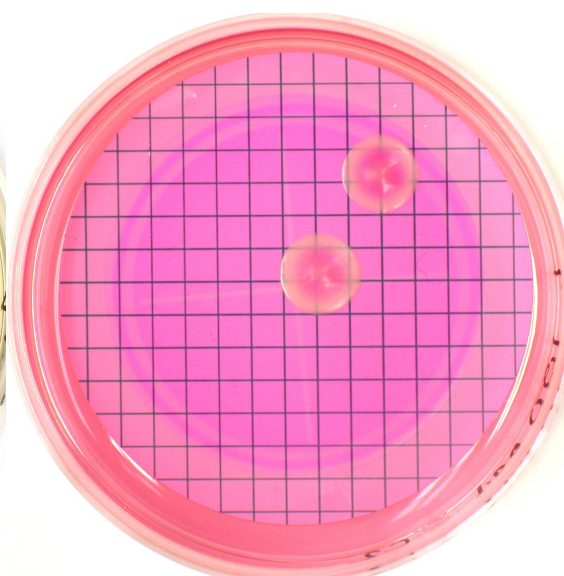


10 ml

Actinomycete Isolation Agar, 25 °C



1 ml, 7 days



100 ml, 7 days

m-RBCC Agar, 25 °C

PT reports published 2014

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

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

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

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

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

PT reports published 2015

Proficiency Testing – Food Microbiology, January 2015

Proficiency Testing – Drinking Water Microbiology, March 2015, by Tommy
Šlapokas

Proficiency Testing – Food Microbiology, April 2015

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, 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