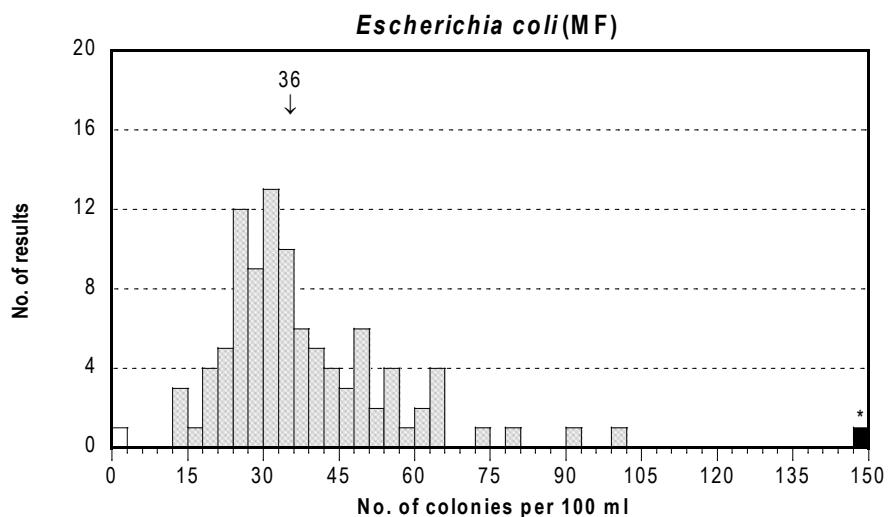


## Proficiency Testing

# Drinking Water Microbiology

## 2012:1, March

by Tommy Šlapokas, Malin Lindqvist and Kirsi Mykkänen





*Proficiency Testing*

# Drinking Water Microbiology

2012:1, March

*Tommy Šlapokas*<sup>1</sup>

*Malin Lindqvist*<sup>2</sup>

*Kirsi Mykkänen*<sup>2</sup>

<sup>1</sup> Compilation and writing <sup>2</sup> Laboratory work

2<sup>nd</sup> edition

Microbiology Division  
National Food Agency  
Box 622  
SE-751 26 UPPSALA  
SWEDEN

Uppsala 2012



*Edition*

Version 2 (10 December 2012)

*Editor in chief*

Annika Rimland, Head of the Science Department, National Food Agency

*Responsible for the scheme*

Tommy Šlapokas, Microbiologist, Microbiology Division, National Food Agency

# Contents

<b>Introduction .....</b>	5
<b>Design .....</b>	5
- Analyses and mixtures .....	5
- Quality control of the mixtures .....	7
<b>Laboratory results .....</b>	8
- General information regarding the results .....	8
- Outcome of the mixtures .....	9
Mixture A .....	9
Mixture B .....	16
Mixture C .....	21
<b>Outcome of the methods .....</b>	26
- Method information by use of internet .....	26
- General information regarding methods outcome .....	26
- Coliform bacteria and <i>E. coli</i> with membrane filtration methods (MF) .....	26
Alternative methods .....	26
Results .....	28
- <i>Clostridium perfringens</i> with membrane filtration methods (MF) .....	31
Various methods .....	31
Results .....	31
- Moulds and yeasts in water (MF) .....	33
Various methods .....	33
Results .....	33
<b>The outcome of deviating results – assessment .....</b>	36
<b>Figure 2 — Box plot .....</b>	38
<b>References .....</b>	41
<b>Annex A — all analytical results .....</b>	42
<b>Annex B— z-values to use in the follow-up process .....</b>	46
<b>Annex C — photo example of colony appearance on some media .....</b>	50



## Introduction

All analytical activities require the execution of 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 examined by a number of laboratories. The laboratories must follow instructions, perform analyses on the samples provided and report their results to the organiser. They are also expected to use their routine methods for their analyses. The organiser subsequently evaluates the results using statistical tools and finally compiles them in a report.

### ***Benefits of the National Food Agency's proficiency tests***

1. Laboratories are externally evaluated with respect to their analytical competence, including usage of methods, documentation and orderliness.
2. Accreditation bodies are provided with a tool for inspections regarding new accreditation or maintenance of accreditation.
3. Laboratories and the organiser improve their knowledge of the efficiency of analytical methods used routinely by participating laboratories with respect to various types of organisms.

## Design

### **Analyses and mixtures**

This proficiency test was performed in March 2012, and is registered as no. 610/2012 at the National Food Agency, Uppsala.

Samples were sent to 99 laboratories 33 of which were in Sweden, 59 in other Nordic countries and 7 in other countries. One laboratory did not report results.

### ***Assessed parameters***

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

**Coliform bacteria** and ***Escherichia coli*** with rapid kit methods using most probable numbers (MPN)

**Presumptive *Clostridium perfringens*** with MF, colonies before confirmation

***Clostridium perfringens*** with MF

**Microfungi (yeasts and moulds)** with MF

**Culturable microorganisms (total count)** after incubation for 3 days at **22±2 °C**

### ***Not assessed parameters***

For the analyses using membrane filtration, the number of **suspected colonies** obtained on the initial culture plates could be reported by the participants, i.e. before the confirmation steps. However, these results are not included in the

calculation of deviant results but are used as information for interpretation and discussion of analyses outcomes.

The proficiency test comprised three simulated water samples. Each laboratory was assigned to perform the analyses according to its methods routinely used on drinking water samples. The test material is first and foremost adjusted to the EN ISO methods for analyses of drinking water, stated in the drinking water directive of the European Union (1). Accepted alternative methods in EU are also possible to use, as well as other similar methods.

Three freeze-dried test materials were produced with different microorganism mixtures. The material was manufactured and freeze-dried in portions of 0.5 ml in small vials, according to the description by Peterz and Steneryd (2). Each

**Table 1** Microbial mixtures<sup>1</sup>

Mixture	Microorganisms	Strain no.	No. of cfu/100 ml <sup>2</sup>
A	<i>Escherichia coli</i>	SLV-084	180
	<i>Serratia marcescens</i>	SLV-040	400
	<i>Clostridium perfringens</i>	SLV-442	370
	<i>Phoma glomerata</i>	SLV-543	20
	<i>Stenotrophomonas maltophilia</i>	SLV-041	7*
B	<i>Escherichia coli</i>	SLV-165	15
	<i>Aeromonas hydrophila</i>	SLV-533	160
	<i>Issatchenkia orientalis</i>	SLV-498	450
	<i>Phialophora fastigiata</i>	SLV-504	85
	<i>Staphylococcus cohnii</i>	SLV-462	78*
C	<i>Klebsiella pneumoniae</i>	SLV-186	580
	<i>Klebsiella oxytoca</i>	SLV-089	950
	<i>Clostridium bifermentans</i>	SLV-009	(260) <sup>#</sup>
	<i>Candida glabrata</i>	SLV-052	800

1 The links between the mixtures and the randomised sample numbers are shown in Annex A

2 Results based on duplicate analyses of 10 vials per mixture, performed at the National Food Agency (Table 2); m-Endo Agar LES was used for *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. marcescens* and *A. hydrophila*; TSC Agar for *C. perfringens* and *C. bifermentans*; RBCC Agar for *Ph. glomerata*, *I. orientalis*, *Ph. fastigiata* and *C. glabrata*; YeA for *S. maltophilia* and *S. cohnii* – cfu = colony forming units

\* cfu per ml

# uncertain value

laboratory received one vial of each mixture. The simulated water samples were prepared by dissolving the content of the vials in 800 ml of sterile diluent. The composition of each mixture is listed in **Table 1**.

### Quality control of the mixtures

It is essential to have a homogeneous mixture and a uniform volume in all vials in order to allow comparison of all freeze-dried samples derived from one mixture. The volume was checked in at least 11 vials of each mixture and the biggest differences between vials were 5, 4 and 5 mg for mixture A, B and C, respectively. The highest accepted volume variation is 15 mg (3%). **Table 2** presents the coefficients of variation (CV) of the results from duplicate analyses of 10 vials from each mixture. The results relate to the unit by volume at which the colonies were counted. The highest accepted CV normally is 25%. For very low colony counts a higher CV is accepted. However, no such analyses were present this time. Hence, according to the criteria, the three mixtures are homogenous. For a definition of low colony count and more about the calculations, see the scheme protocol (3)

**Table 2** Coefficients of variation (%; square root transformed results<sup>1</sup>) for various microbial groups, in analyses performed in connection to the proficiency test

Analysis	Mixture		
	A	B	C
Suspected coliform bacteria (MF) <sup>2</sup>	4 <sup>a</sup>	3	3 <sup>b</sup>
Suspected thermotolerant colif. bact. (MF) <sup>3</sup>	10 <sup>a</sup>	12	7 <sup>b</sup>
Presumptive <i>Clostridium perfringens</i> <sup>4</sup>	12 <sup>a</sup>	—	—
Moulds (MF) <sup>5</sup>	11	15 <sup>a</sup>	—
Yeasts (MF) <sup>5</sup>	—	5 <sup>a</sup>	5 <sup>b</sup>
Culturable microorg., 3d 22 °C (pour-plate) <sup>6</sup>	14	4	4

1 n=10 mean values á 2 analyses of 100 ml for MF and 1 ml for pour-plate, if other is not stated; mixtures A, B and C analysed 9, 7 and 5 weeks ahead of the proficiency test, respectively

2 m-Endo Agar LES according to SS 028167 [a preliminary analysis of concentrations was also done on Lactose TTC Agar with Tergitol according to SS-EN ISO 9308-1:2000]

3 m-FC Agar, 44 °C according to SS 028167 [a preliminary analysis of concentrations was also done on Lactose TTC Agar with Tergitol according to SS-EN ISO 9308-1:2000]

4 Spores + Vegetative cells; Tryptose Sulphite Cycloserine Agar (TSC) 44 °C according to ISO/CD 6461-2:2002

5 Rose Bengal Agar containing both chlortetracycline and chloramphenicol (RBCC) according to SS 028179

6 Yeast extract Agar (YeA; yeast extract agar with tryptone) according to SS-EN ISO 6222:1999

a Results for 10 ml

b Results for 5 ml

— Not analysed

## Laboratory results

### General information regarding the results

The histograms (**Figure 1**) show the actual distribution of the results. False positives are not presented in histograms but are compiled in **Table 3** together with the other results with annotations. All reported laboratory results are listed in **Annex A**. Z-values for the all evaluated results are shown in **Annex B** and pictures of colony appearance on various media are presented in **Annex C**.

Most histograms have “tails” in either or both directions, due to values that do not belong to a normal distribution. Calculations are performed after square root transformations of the results which give better normal distributions and therefore decrease the significance of the “tails”. Very deviating values are present in most analyses and are identified as outliers (black bars) with the aid of Grubbs’ test according to a modification by Kelly (4). A level of 1% is used as risk to incorrectly assess a result as being an outlier. Although the method is objective, it is a prerequisite that the results are normally distributed in order to obtain correct outliers. 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 negative results are presented with white bars in the histograms. False results and outliers are not included in the calculations. Calculations are more elaborately described in the scheme protocol (3).

The coefficient of variation (CV) is used to measure the dispersion of the laboratory results. If 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.

**Table 3** Number of analytical results with annotation in evaluated analyses

Classification of results	Number of results				No. of laboratories
	A	B	C	Total	
No. of evaluated results	526	527	528	1581	98 <sup>a</sup>
False positives	9	1	12	22	21
False negatives	1	27	1	29	26
Low outliers	5	2	2	9	5
High outliers	7	11	2	20	17
No. of results with annotation	22	41	17	80	52 <sup>b</sup>

a Number of laboratories that reported analytical results

b Number of laboratories that reported at least one result with annotation

## Outcome of the mixtures

### Mixture A

General information about the mixture

The composition of mixture A is presented in **Table 1**. The analyses for the detection of each microorganism are listed in **Table 4**, as well as the results average and the percentage of deviant results.

**Table 4** Outcome of analyses for mixture A; F+ and F- are % of false positive and false negative results, respectively. Outl < and Outl > are % of low and high outliers, respectively. Shaded analyses are not numerically assessed and the median is stated instead of mean.

Analysis	Organisms	cfu/ vol <sup>1</sup>	CV <sup>2</sup> (%)	F+	F-	Outl <	Outl >
Susp. coliform bacteria (MF)	<i>E. coli</i> { <i>S. marcescens</i> }	170	—				
Coliform bacteria (MF)	<i>E. coli</i> { <i>S. marcescens</i> }	196	27	-	0	0	0
Susp. thermotol. colif. bact. (MF)	<i>E. coli</i>	120	—				
<i>E. coli</i> (MF)	<i>E. coli</i>	145	13	-	0	0	0
Coliform bact. (rapid method)	<i>E. coli</i> <i>S. marcescens</i>	406	15	-	0	3	2
<i>E. coli</i> (rapid method)	<i>E. coli</i>	160	10	-	0	5	2
Presumptive <i>C. perfringens</i> (MF)	<i>C. perfringens</i>	446	31	-	0	0	0
<i>C. perfringens</i> (MF)	<i>C. perfringens</i>	327	38	-	3	0	0
Moulds (MF)	<i>Ph. glomerata</i>	11	15	-	0	0	5
Yeast (MF)	—	0	-	9	-	-	-
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>S. maltophilia</i> <i>S. marcescens</i> <i>E. coli</i>	12	21	-	0	0	3

1 "colony forming units" per unit of volume – 1 ml for total count microorg., otherwise 100 ml

2 "Coefficient of Variation" – calculated from square root transformed results (see Annex A)

- numerical value impossible to obtain

— organism absent or numerical value has not been calculated

( ) the organism contributes with very few colonies

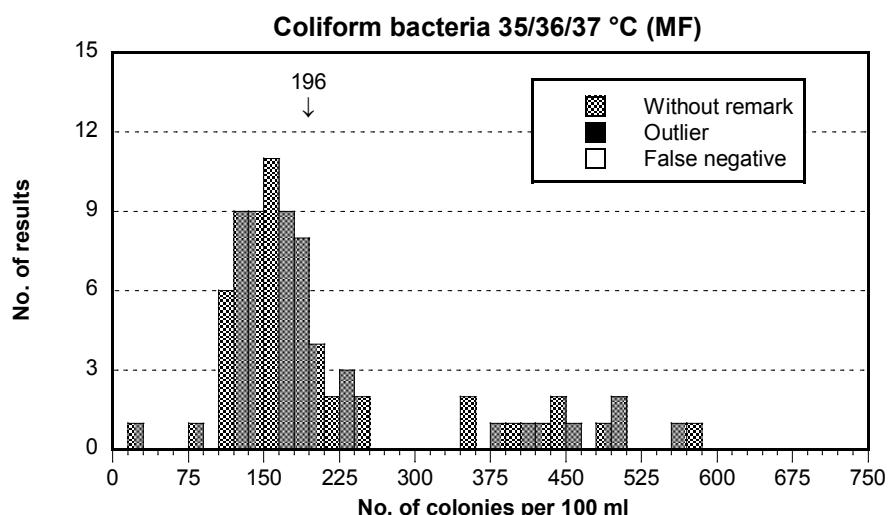
[ ] the organism is false positive in a presumptive analysis

{ } the result depends on the definition

### Coliform bacteria, MF

- The results are quite well distributed but a second minor peak appears centred around values twice as high (**Figure 1A**). This distribution generated a medium CV for the overall results.

- When we performed analyses of the mixture A on m-Endo Agar LES and Lactose TTC Agar, colonies of *S. marcescens* could not be misinterpreted as colonies of coliform bacteria (**Annex C**). Therefore, the concentration of coliform bacteria in mixture A corresponds to the concentration of *E. coli*, 180 cfu/100 ml for our analysis (**Table 1**).
- Results included in the minor peak in the histogram (values >300 cfu/100 ml) are certainly due to counting *S. marcescens* colonies as coliform bacteria. The mean and variation for all results are given in **Table 4**.
- *S. marcescens* does not ferment lactose and therefore should not be considered as coliform bacterium according to the standard methods based on this feature. Hence, values included in the minor peak should be evaluated as high outliers when standard methods using for example m-Endo Agar LES and Lactose TTC Agar, are used. With method based on other detection's mean and therefore defining coliform bacteria in another way, like Chromocult Coliform Agar<sup>®</sup>, colonies of *S. marcescens* can grow and be interpreted as coliform bacteria. See further in the section Outcome of the methods.
- In previous PT rounds with *S. marcescens* and *E. coli* in the same mixture, some high results were obtained similar to those in this round (9, 10)

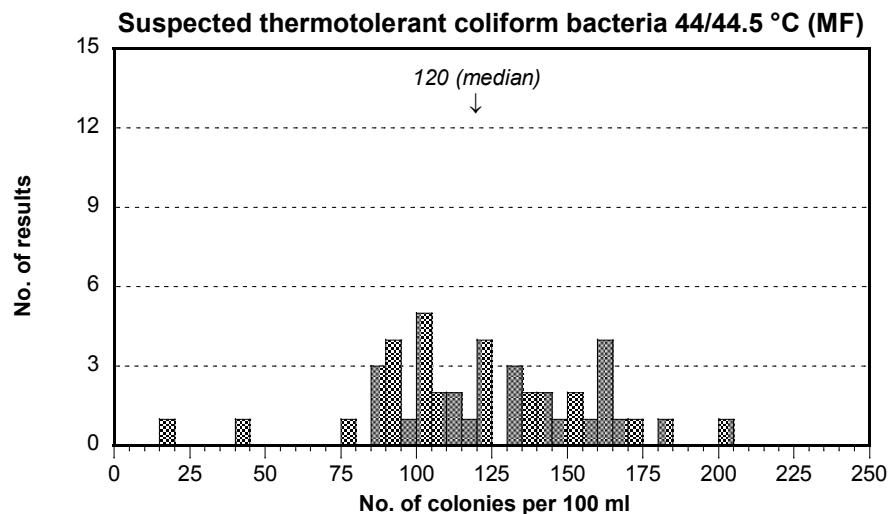


**Figure 1A Mixture A, Histogram of all analytical results.** False negatives are presented as white bars. Outliers, false negatives excluded, are represented by black bars. The x-axis scale is not adjusted to very high deviating results. They are marked with an asterisk. The mean value of the analysis is stated above the bars. Calculations have been made from square root transformed results, outliers and false negatives excluded.

Suspected thermotolerant coliform bacteria, MF

- Results are well distributed except for few low values (**Figure 1B**).

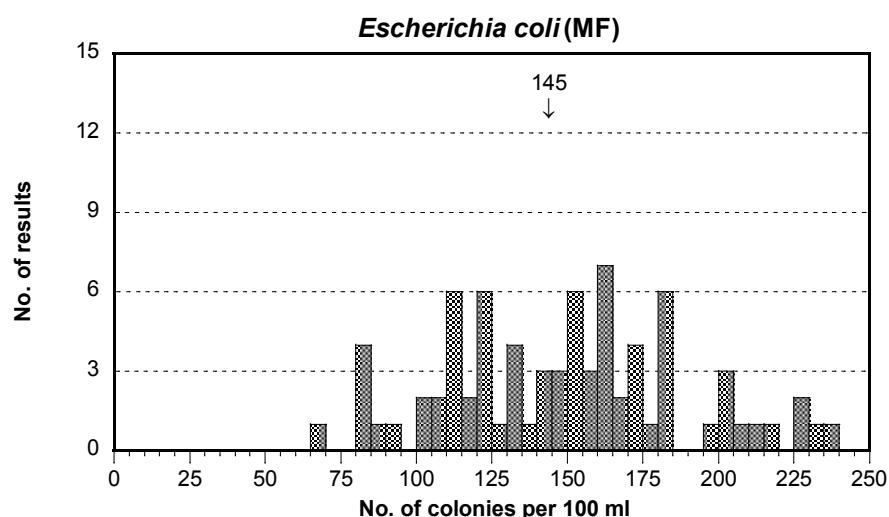
- There are no outliers or false results for this analysis as it is not evaluated.
- The results correspond approximately to the major peak of the histogram for coliform bacteria (**Figure 1A**), that is the concentration of *E. coli*. However the results are from various medium at 44/44.5 °C, which gives often lower values than analyses performed at 36±2 °C.



**Figure 1B** *Mixture A*, see figure 1A for explanation

*E. coli*, MF

- The results are well distributed (**Figure 1C**) with a small dispersion.

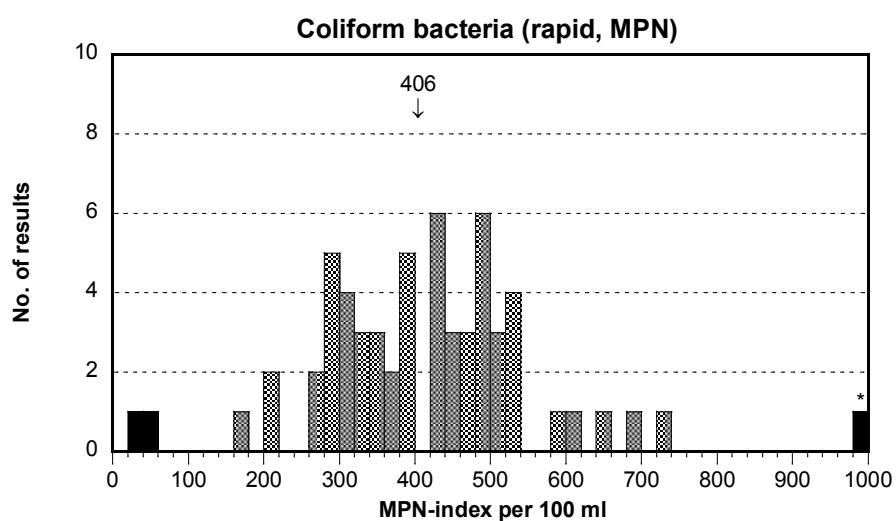


**Figure 1C** *Mixture A*, see figure 1A for explanations

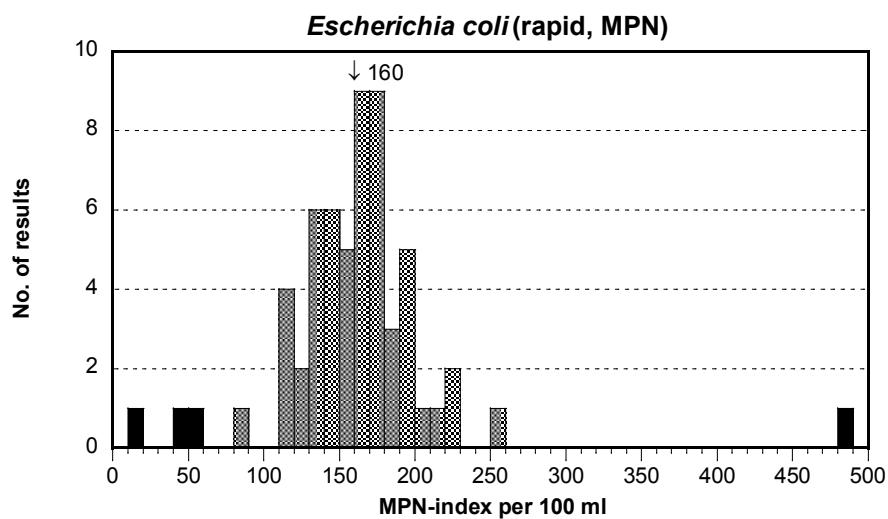
- The histogram includes values obtained on different media at 35/36/37 °C or 44/44.5 °C after confirmation. The results correspond in principle to the major peak in the histogram for coliform bacteria (**Figure 1A**).

Coliform bacteria, rapid method (MPN)

- The results are well distributed with a single peak (**Figure 1D**). The dispersion is small.
- Results correspond approximately to the minor peak in the histogram for coliform bacteria obtained with MF methods (**Figure 1A**) and include both the colonies of *E. coli* and *S. marcescens*.



**Figure 1D Mixture A**, see figure 1A for explanations



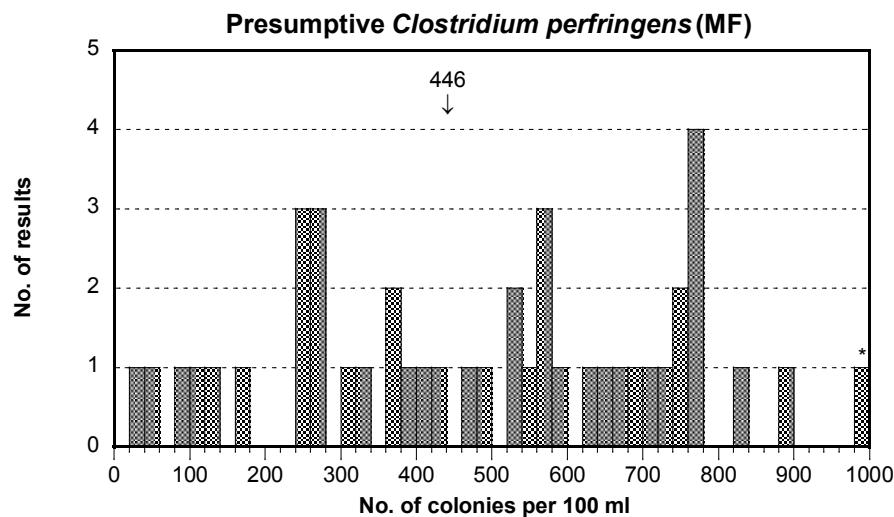
**Figure 1E Mixture A**, see figure 1A for explanations

### E. coli, rapid method (MPN)

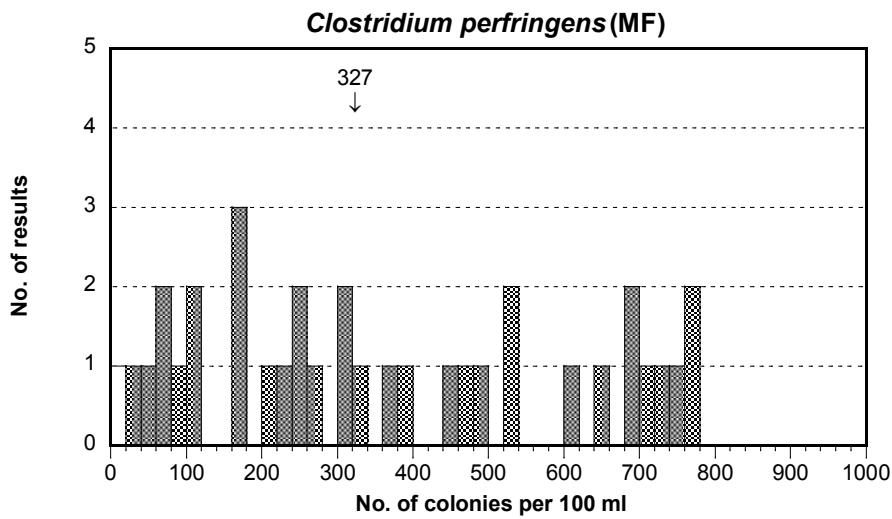
- The results were well distributed and the dispersion was somehow smaller than for the results obtained with MF methods (**Figure 1E**). The dispersion was very small to small.
- Results reflect the number of *E. coli* bacteria in the mixture with an average value slightly higher than the one obtained with the MF methods.

### Presumptive and confirmed *Clostridium perfringens*, MF

- For both analyses the distributions of the results are really bad with large dispersions in both cases (**Figure 1F and 1G**).
- No outliers could be evaluated due to the large dispersion.
- The *C. perfringens* strain present in the mixture A grows well on TSC Agar even if the colonies can sometimes appear light-coloured. On the opposite, lower recovery often appears on medium like m-CP Agar (see the section Outcome of the methods for further discussion).



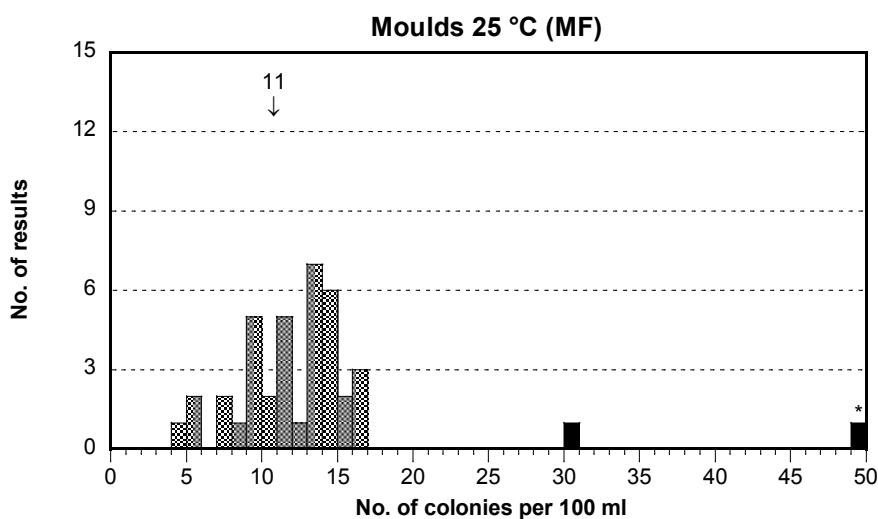
**Figure 1F Mixture A, see figure 1A for explanations**



**Figure 1G Mixture A, see figure 1A for explanations**

#### Moulds and yeasts, MF

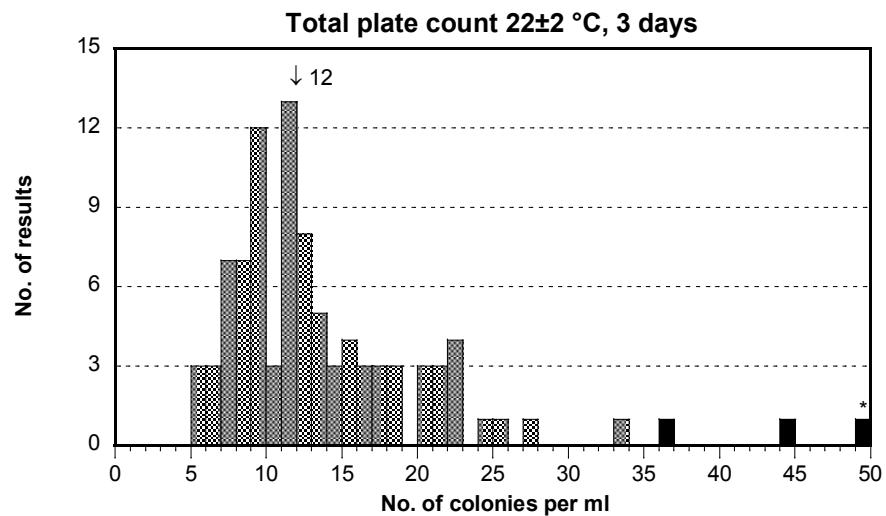
- Mould colonies were formed by *Phoma glomerata*. The distribution of the results is good with a small dispersion (**Figure 1H**).
- No yeasts were present in mixture A, however 9 out of 40 laboratories reported a quite high amount of yeast colonies which exclude the possibility of contamination from the air. By microscopy it appears evident that the reddish colonies don't originate from yeast cells but bacterial cells. Biochemical identification revealed that it is the strain of *Serratia marcescens* that forms these colonies. Thus, the results from the 9 laboratories that reported yeast are therefore considered as false positive.



**Figure 1H Mixture A, see figure 1A for explanations**

Culturable microorganisms 22 °C, 3 days

- The results were well distributed (**Figure 1I**). The dispersion was medium and not smaller due to the low number of colonies.
- The results included about equal colony numbers of coliform bacteria and *Stenotrophomonas maltophilia*. The analysis was without problem.



**Figure 1I Mixture A**, see figure 1A for explanations

### **Mixture B**

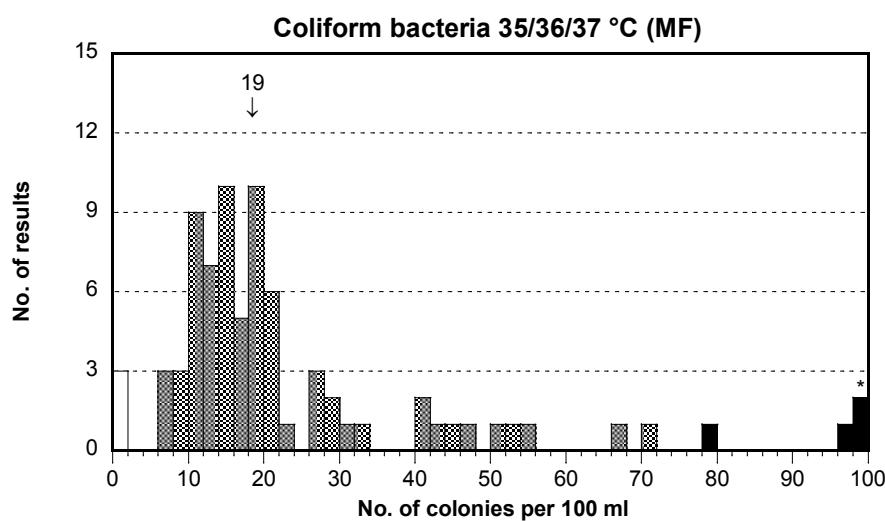
General information about the mixture

The composition of mixture B is presented in **Table 1**. The analyses for the detection of each microorganism are listed in **Table 5**, as well as the results average and the percentage of deviant results.

**Table 5** Outcome of each analysis for mixture B; see Table 4 for explanations.

Analysis	Organisms	cfu/ vol <sup>1</sup>	CV <sup>2</sup> (%)	F+	F-	Outl <	Outl >
Susp. coliform bacteria (MF)	<i>E. coli</i> <i>[A. hydrophila]</i>	121	—				
Coliform bacteria (MF)	<i>E. coli</i>	19	30	-	4	0	5
Susp. thermotol. colif. bact. (MF)	<i>E. coli</i>	10	—				
<i>E. coli</i> (MF)	<i>E. coli</i>	14	24	-	4	0	5
Coliform bact. (rapid method)	<i>E. coli</i>	15	13	-	0	0	2
<i>E. coli</i> (rapid method)	<i>E. coli</i>	15	13	-	0	0	0
Presumptive <i>C. perfringens</i> (MF)	—	0	-	2	-	-	-
<i>C. perfringens</i> (MF)	—	0	-	0	-	-	-
Mould (MF)	<i>Ph. fastigiata</i>	291	42	-	10	0	0
Yeast (MF)	<i>I. orientalis</i>	518	8	-	44	0	5
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>S. cohnii</i> <i>(A. hydrophila)</i> <i>(E. coli)</i>	81	8	-	0	2	0

Coliform bacteria, MF

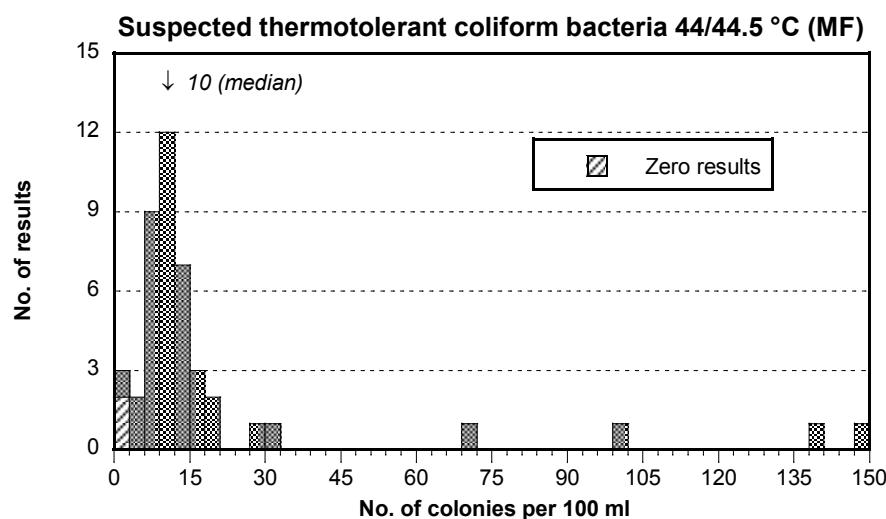


**Figure 1J** *Mixture B*, see figure 1A for explanations

- The results are spread with a tail of higher values which generated a medium dispersion close to large (**Figure 1J**).
- Coliform bacteria are represented by a typical strain of *E. coli* in this mixture.
- Nine laboratories reported identical results for the analysis of suspected and confirmed coliform bacteria. In some cases the strain *A. hydrophila* seems to have been counted as a coliform bacterium because confirmation step was not performed or failed. In other cases the colonies of *A. hydrophila* were probably not taken into account even in the amount of suspected coliform bacteria. In most cases *A. hydrophila* were excluded after the confirmation step and therefore the median value decreased from 121 to 17 cfu/100 ml (**Annex A**).

#### Suspected thermotolerant coliform bacteria, MF

- Most results are gathered but some unexpected high values were also reported, which gives somewhat a scatter of the overall outcome (**Figure 1K**).
- There are no outliers or false results for this analysis as it is not evaluated.
- The results include the amount of *E. coli* colonies on various media after incubation at 44/44.5 °C. This temperature leads often to lower values than the corresponding ones from 35/36/37 °C.

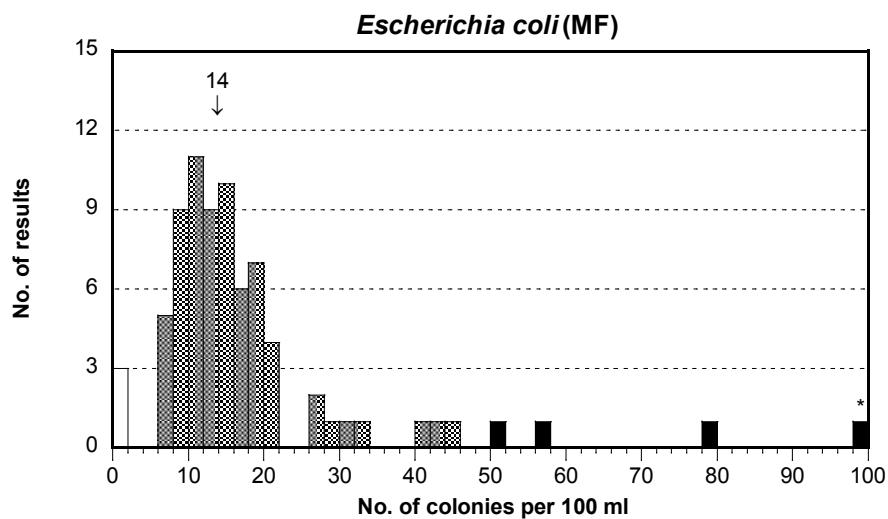


**Figure 1K Mixture B, see figure 1A for explanations**

#### *E. coli*, MF

- The results are a little bit spread with a tail of higher values (**Figure 1L**) and medium dispersion.
- The reason for these high values is not known, even if high results were seen also for coliform bacteria that probably were counted on the same plates. The amount of *A. hydrophila* colonies might have been included in the coliform

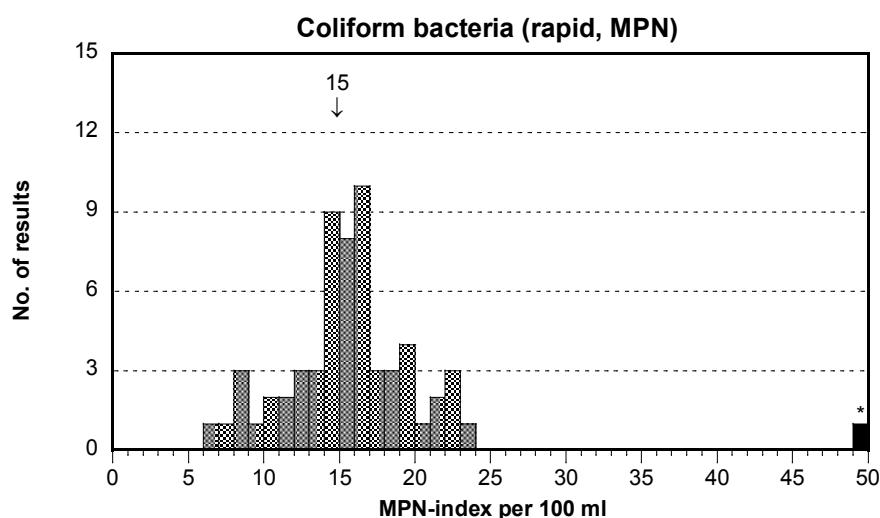
results. However this should not happen after the confirmation steps for analysis of *E. coli*. Some of the high results, therefore seem to come from the high results of suspected thermotolerant coliform bacteria.



**Figure 1L Mixture B**, see figure 1A for explanations

Coliform bacteria, rapid method (MPN)

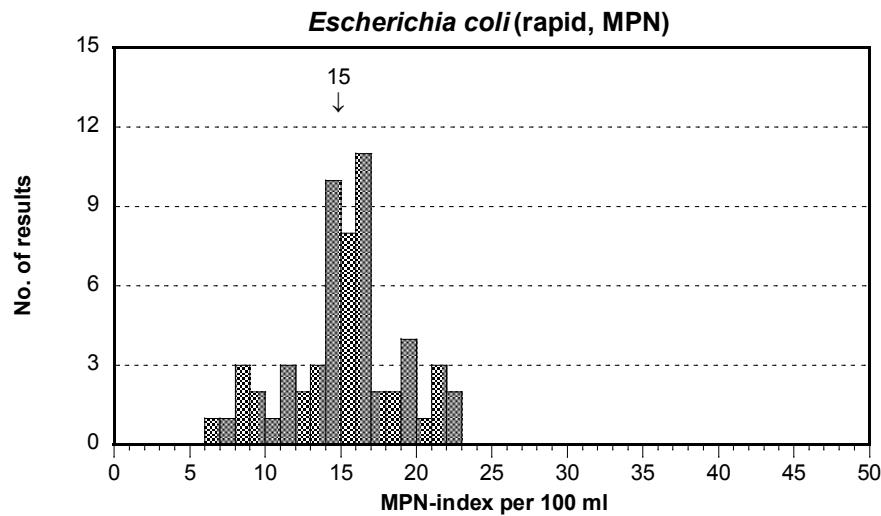
- The distribution of results is good with a small dispersion (**Figure 1M**).
- Often the average values are somewhat higher with Colilert®-18/24 Quanti-Tray® than with MF methods but this time the opposite occurred. It can be explained by the amount of *A. hydrophila* colonies that were included in the counting of the MF methods and there increased the average result.



**Figure 1M Mixture B**, see figure 1A for explanations

E. coli, rapid method (MPN)

- The distribution of the results is good with a small dispersion (**Figure 1N**).
- The results were in principle identical to the results of coliform bacteria analysis with rapid method. In general both parameters are measured with the same kit.



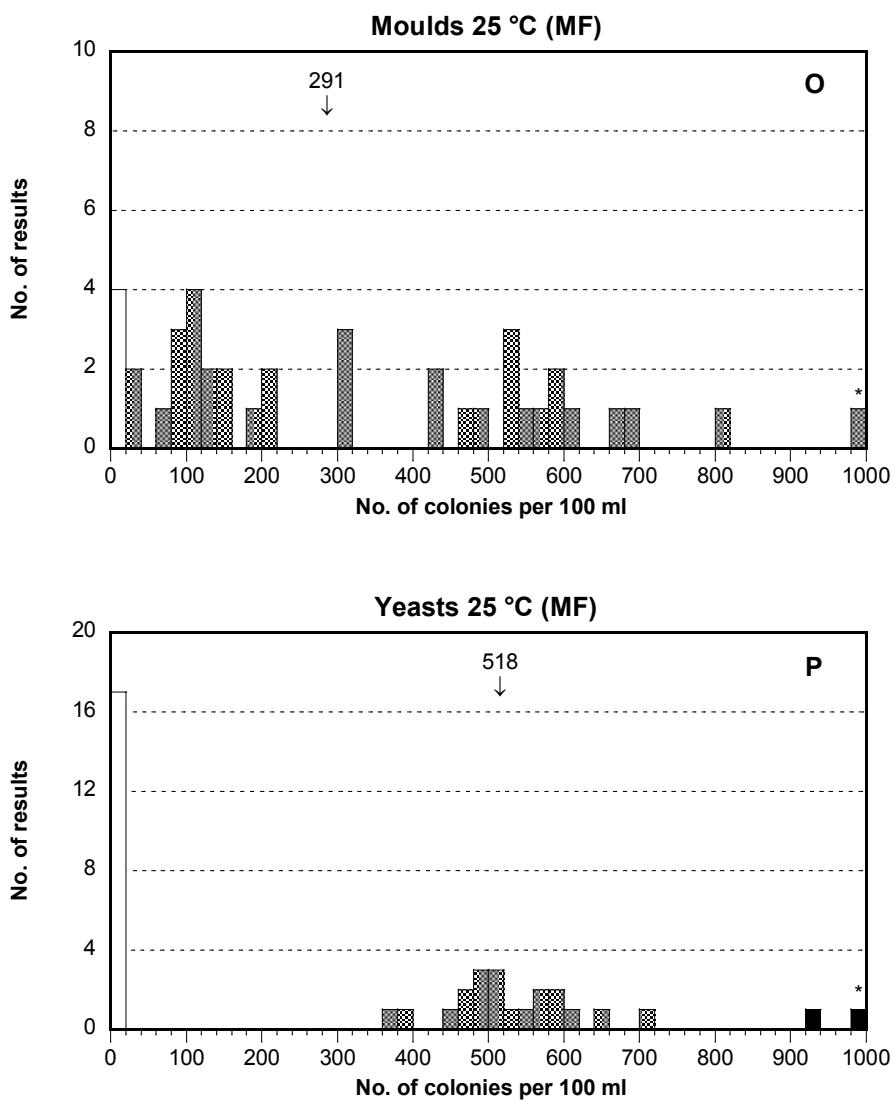
**Figure 1N Mixture B**, see figure 1A for explanations

Presumptive and confirmed *Clostridium perfringens*, MF

No *C. perfringens* were present in mixture B.

Moulds and yeasts, MF

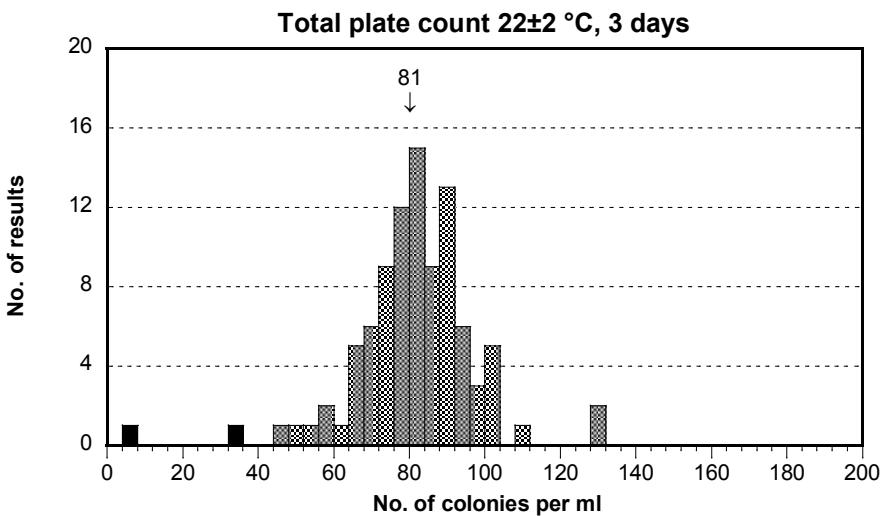
- The results for moulds are mainly divided in two groups: values <220 and >420 cfu/100 ml (3 results are in between) (**Figure 1O**). Therefore the dispersion is very large (42%).
- The results for yeasts are well distributed with a small dispersion, but 17 false negative results were reported (**Figure 1P**).
- The very large dispersion of the mould results is due to a misinterpretation of yeast colonies, including them in values above 300 or 400 cfu/100 ml for moulds and leading to negative results in the yeast analysis.
- The yeast *Issatchenkovia orientalis* forms a kind of filaments (pseudohyphae) appearing similar to hyphae by microscopic observation, which can lead to a misinterpretation of a mould. However, the cells present also more typical yeast structures. *I. orientalis* is the asexual form of the yeast *Candida krusei*.



**Figure 1O and 1P Mixture A**, see figure 1A for explanations

Culturable microorganisms, 22 °C, 3 days

- The results are well distributed with a very small dispersion (**Figure 1Q**).
- The colonies counted are mainly from *S. cohnii* cells but coliform bacteria can also grow and account for some colonies.



**Figure 1Q Mixture B**, see figure 1A for explanations

### Mixture C

General information about the mixture

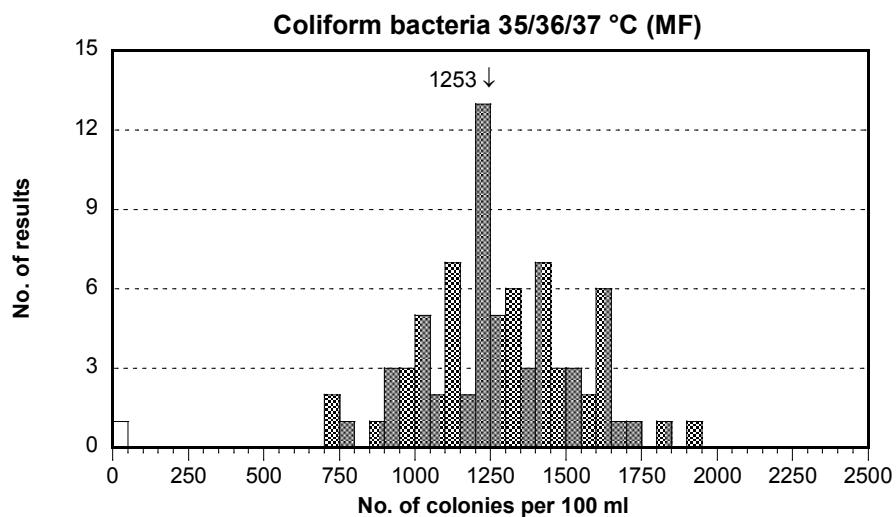
The composition of mixture C is presented in **Table 1**. The analyses for the detection of each microorganism are listed in **Table 6**, as well as the results average and the percentage of deviant results.

**Table 6** The outcome of each analysis in mixture C; see Table 4 for explanations.

Analysis	Organisms	cfu/ vol <sup>1</sup>	CV <sup>2</sup> (%)	F+	F-	Outl <	Outl >
Susp. coliform bacteria (MF)	<i>K. pneumoniae</i>	1245	—				
	<i>K. oxytoca</i>						
Coliform bacteria (MF)	<i>K. pneumoniae</i>	1253	10	-	1	0	0
	<i>K. oxytoca</i>						
Susp. thermotol. colif. bact. (MF)	<i>K. pneumoniae</i>	415	—				
<i>E. coli</i> (MF)		0	-	9	-	-	-
Coliform bact. (rapid method)	<i>K. pneumoniae</i>	1320	10	-	0	3	0
	<i>K. oxytoca</i>						
<i>E. coli</i> (rapid method)		0	-	0	-	-	-
Presumptive <i>C. perfringens</i> (MF) [ <i>C. bifermentans</i> ]		1028	93	-	0	0	0
<i>C. perfringens</i> (MF)		0	-	11	-	-	-
Mould (MF)		0	-	3	-	-	-
Yeast (MF)	<i>C. glabrata</i>	817	8	-	0	0	3
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>K. oxytoca</i>	16	14	-	0	0	1
	<i>C. glabrata</i>						
	<i>K. pneumoniae</i>						

#### Coliform bacteria, MF

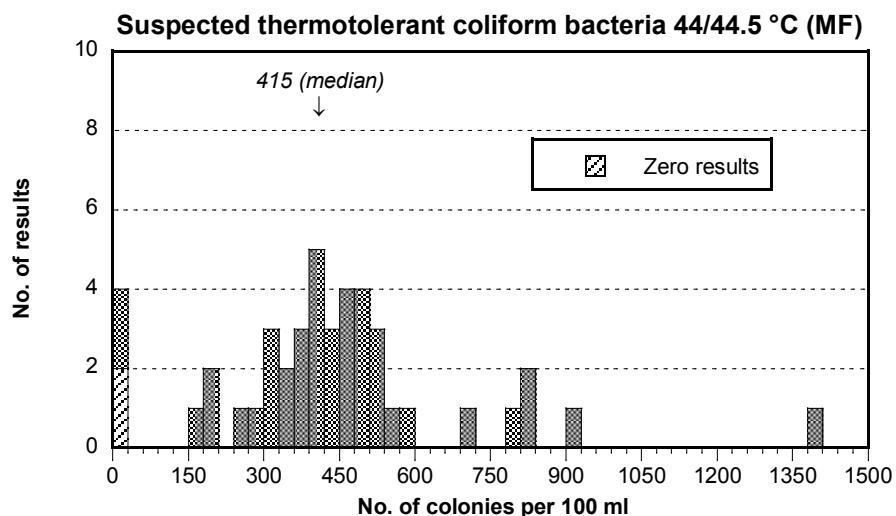
- The distribution of results was good with a very small dispersion (**Figure 1R**).
- The coliform bacteria included in mixture C were *K. pneumoniae* and *K. oxytoca* which form typical colonies on m-Endo Agar LES and LTTC Agar.



**Figure 1R Mixture C**, see figure 1A for explanations

#### Suspected thermotolerant coliform bacteria, MF

42 results were reported for this analysis (**Figure 1S**). Colonies are from *K. pneumoniae* which growth on m-FC agar or Lactose TTC Agar at 44/44.5 °C. The explanation for the two zero values are unknown. This analysis is not evaluated.



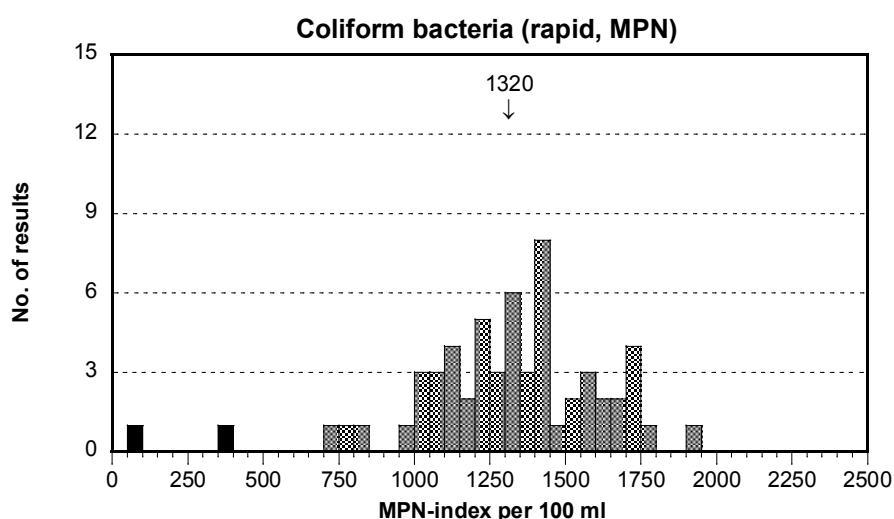
**Figure 1S Mixture C**, see figure 1A for explanations

#### *E. coli*, MF

Mixture C did not contain any *E. coli* strain, however 7 false positive results were reported. Either confirmation steps were not performed on thermotolerant colonies, or confirmation was performed for oxidase and indol production only but on colonies grown at 35-37 °C. Sometimes, *K. oxytoca* which is oxidase negative and indole positive can generate a positive result for indole test in broth with tryptophan at 44 °C (5). Such colonies will therefore be misinterpreted as *E. coli*. If an additional confirmation step is performed, like the production of gas or a  $\beta$ -glucuronidase test (MUG), the false positive results will be avoided.

#### Coliform bacteria, rapid method (MPN)

- The distribution of results is good with a very small dispersion (**Figure T**)
- The results are quite similar to those obtained with MF methods but slightly higher. The same organisms are detected in both analyses, i.e. *K. pneumoniae* and *K. oxytoca*.



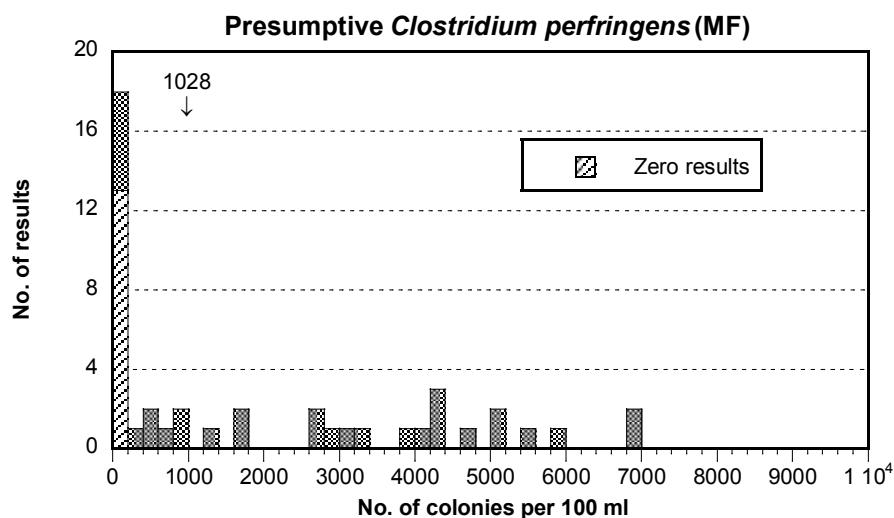
**Figure 1T Mixture C, see figure 1A for explanations**

#### *E. coli*, rapid method (MPN)

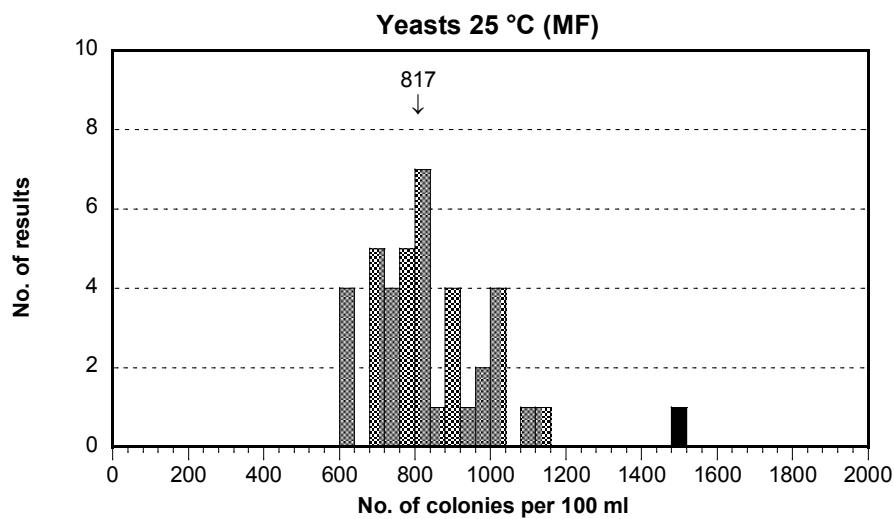
No *E. coli* strain was present and no false positive results were reported.

### Presumptive and confirmed Clostridium perfringens, MF

- Many laboratories reported presumptive *C. perfringens* in mixture C. The distribution of the results is wide without obvious grouping (**Figure 1U**), which generates a very large dispersion (93%).
- For this reason no outliers could be identified but 13 zero values were reported.
- Mixture C contained no *C. perfringens* but a strain of *C. bifermentans* that forms more or less black colonies on TSC Agar. The zero values were mainly obtained with other types of media (see the section Outcome of the methods).



**Figure 1U Mixture C**, see figure 1A for explanations



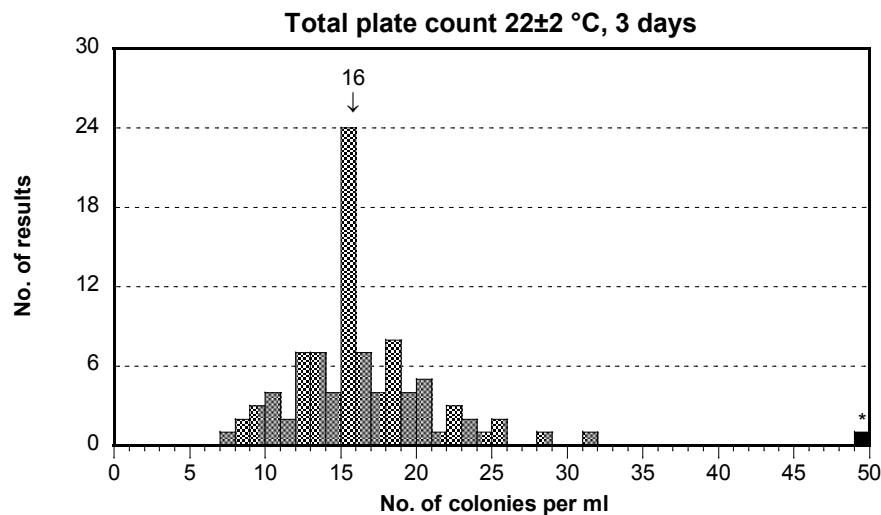
**Figure 1V Mixture C**, see figure 1A for explanations

Moulds and yeasts, MF

- Only the yeast *Candida glabrata* was present in mixture C.
- The dispersion of the results is good with a very small dispersion (**Figure 1V**).

Culturable microorganisms 22 °C, 3 days

- The results were well distributed with a small dispersion (**Figure 1W**).
- The colonies are mainly formed by the coliform bacteria *K. pneumoniae* and *K. oxytoca* and the yeast *C. glabrata*.



**Figure 1W Mixture C**, see figure 1A for explanations

## **Outcome of the methods**

### **Method information by use of internet**

According to EN ISO/IEC 17043, for which the proficiency testing program organized by the National Food Agency is accredited since early 2012, the provider shall be able to group results according to the methods used. The method information is reported via our website [www.slv.se/absint](http://www.slv.se/absint), after logging on.

### **General information regarding methods outcome**

The number of results for the various methods is clear from the descriptive part of **Annex A**. Although method information is available for all numerical results, it is not always easy to interpret. For example, sometimes the medium used differs from what is stated in the standard. Results from such laboratories are usually not shown in this report. They will be omitted or placed in the group "Other".

Method information from laboratories with outliers or false results for a particular analysis will not be included in the compilations. However, it is possible that some methods generate more deviating results than others and therefore might be mentioned in the text. Methods with 3 or fewer results will normally not be discussed in the comparisons.

No statistical tests are done in this report based on results grouped according to method parameters. Thus, significant differences cannot be discussed.

The method outcome of coliform bacteria and *E. coli* obtained with rapid methods as well of culturable microorganisms are not discussed for this PT round.

### **Coliform bacteria and *E. coli* with membrane filtration methods (MF)**

#### ***Alternative methods***

In Norway, Finland, Sweden and some other European countries other membrane filtration methods (MF) for coliform bacteria can be used at statutory sampling, as alternatives to the reference method EN ISO 9308-1:2000 based on Lactose TTC Agar with Tergitol 7 ("LTTC Agar"). National methods in Norway, Finland and Sweden are based on m-Endo Agar LES ("LES endo agar") and m-FC Agar, but are usually more or less modified. In Sweden and Finland, m-FC Agar must not be used for *statutory sampling* of drinking water. Rather *E. coli* should be identified by confirmation from LES Endo Agar plates incubated at 36±2 °C. In Sweden, *E. coli* is confirmed by a negative oxidase test for coliform bacteria, a positive indole test at 44 °C and a positive β-glucuronidase activity test. In Finland, an additional gas test at 44 °C or β-glucuronidase activity test is recommended as complement to the indole test. To be interpreted as *E. coli*, colonies must there be indole positive as well as gas or β-glucuronidase positive. The β-glucuronidase test is a complement to the indol test in order to eliminate for example indole positive and, in confirmation broth, thermotolerant strains of *Klebsiella oxytoca* (5).

**Table 7** Numbers of results and their outcome, outliers excluded, with different method standards for the analysis of coliform bacteria (A) and *E. coli* (B) with MF methods and incubation at 36±2 °C

Method standard	Total no. of res.	Mixture					
		A		B		C	
		n	Mv <sup>1</sup>	n	Mv <sup>1</sup>	n	Mv <sup>1</sup>
<b>A. Coliform bacteria</b>	<b>87</b>	<b>79</b>	<b>196</b>	<b>71</b>	<b>19</b>	<b>78</b>	<b>1253</b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	24	20	190	16	23	20	1141
SS 028167 <sup>b</sup>	25	23	161	22	16	22	1295
SFS 3016 <sup>c</sup>	27	26	253	24	18	26	1322
NS 4788 <sup>d</sup>	6	6	160	6	23	6	1219
Other	5	4	149	3	26	4	1203
<b>B. Escherichia coli</b>	<b>52</b>	<b>49</b>	<b>155</b>	<b>44</b>	<b>15</b>	<b>45</b>	<b>0</b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	14	12	149	10	15	10	0
SS 028167 Modif. <sup>b, e</sup>	17	17	161	17	15	17	0
SFS 3016/4088 Modif. <sup>e, f, g</sup>	18	17	149	15	14	16	0
NS 4792 <sup>h</sup>	2	2	145	1	15	1	0
Other	1	1	236	1	18	1	0

<sup>1</sup> Mean values based on square root transformation; cfu per 100 ml

- a ISO/CEN Standard: Water quality — Detection and enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method, September 2000 (XX stands for the national translations, if any)
- b Swedish Standard: Coliform Bacteria, Thermotolerant Coliform Bacteria and *Escherichia coli* in Water — Determination with Membrane Filtration Method (MF), 2<sup>nd</sup> ed. 1996-03-13
- c Finnish Standard: Membrane filter technique for the enumeration of total coliform bacteria in water, 2001-05-21
- d Norwegian Standard: Coliform Bacteria — Membrane filter method, 1<sup>st</sup> ed. May 1990
- e *E. coli* are coliform bacteria from m-Endo Agar LES that are indole positive at 44 °C and also β-glucuronidase positive
- f Finnish Standard: Membrane filter technique for the enumeration of thermotolerant (faecal) coliform bacteria in water, 2001-05-21
- g *E. coli* are coliform bacteria from m-Endo Agar LES that are indole positive, alternatively gas & indole positive or β-glucuronidase & indole positive at 44 °C
- h Norwegian Standard: Thermotolerant coliform bacteria and presumptive *E. coli* — Membrane filter method, 1<sup>st</sup> ed. May 1990

Apart from the reference method XX-EN ISO 9308-1:2000 (XX stands for the national versions), the older national standards used in Finland, Norway and Sweden are presented individually in **Table 7** and **8**. For *E. coli*, the terms SS 028167 Modif. and SFS 3016/4088 Modif. include modifications such as e.g. those that were stated above, with respect to Sweden and Finland.

## Results

Regarding coliform bacteria, there is no general difference between methods for mixture C (**Table 7A**) which contained two typical coliform bacteria strains easy to interpret (**Annex C**). On the other hand, with mixture A, the results obtained using the Swedish or Norwegian standard methods were lower than those obtained when using Finnish standard method or the reference method XX-EN ISO 9308-1. This is certainly linked to the media actually used, the way to interpret colony appearance and possibly the time of incubation. *S. marcescens* forms atypical (**Annex C**). On chromogenic media like Chromocult Coliform Agar®, *S. marcescens* appears like coliform bacteria. Possibly, colonies may be interpreted as colonies on media based on lactose fermentation after one day of incubation from

**Table 8** Numbers of results and their outcome with different method standards for the analysis of suspected thermotolerant coliform bacteria (A; median of all results) and *E. coli* (B; mean outliers excluded) with MF methods and incubation at 44/44.5 °C

Method standard	Total no. of res.	Mixture					
		A		B		C	
		n	Mv <sup>1</sup>	n	Mv <sup>1</sup>	n	Mv <sup>1</sup>
<b>A. <i>Susp. thermotol. colif. bact.</i></b>	<b>60</b>	<b>44</b>	<b>120</b>	<b>44</b>	<b>10</b>	<b>44</b>	<b>415</b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	10	8	139	8	16	8	403
SS 028167 <sup>b</sup>	13	11	130	11	11	11	430
SFS 4088 <sup>c</sup>	19	13	120	13	10	13	450
NS 4792 <sup>d</sup>	8	7	109	7	8	7	400
Other	10	5	100	5	27	5	410
<b>B. <i>Escherichia coli</i></b>	<b>11</b>	<b>11</b>	<b>132</b>	<b>11</b>	<b>12</b>	<b>11</b>	<b>0</b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	2	2	123	2	13	2	0
SS 028167 <sup>b</sup>	1	1	140	1	14	1	0
SFS 4088 <sup>c</sup>	3	3	153	3	11	3	0
NS 4792 <sup>d</sup>	3	3	105	3	8	3	0
Other	2	2	152	2	21	2	0

<sup>1</sup> Median or mean values based on square root transformation; cfu/100 ml.

- a ISO/CEN Standard: Water quality — Detection and enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method, September 2000 (XX stands for the national translations, if any)
- b Swedish Standard: Coliform Bacteria, Thermotolerant Coliform Bacteria and *Escherichia coli* in Water — Determination with Membrane Filtration Method (MF), 2<sup>nd</sup> ed. 1996-03-13
- c Finnish Standard: Membrane filter technique for the enumeration of thermotolerant (faecal) coliform bacteria in water, 2001-05-21
- d Norwegian Standard: Thermotolerant coliform bacteria and presumptive *E. coli* — Membrane filter method, 1<sup>st</sup> ed. May 1990

coliform bacteria after two days of incubation on m-Endo Agar LES. The reason for the high values obtained with Finnish standard method is not clear.

Some method differences occurred also in mixture B. In this case, it is the used Norwegian standard method, as well as some other methods, together with the reference method XX-EN ISO 9308-1 that gave higher results than the Finnish and Swedish standard methods. Mixture B contained a strain of *Aeromonas hydrophila* which forms coliform-like colonies on media based on lactose fermentation. The result differences depend certainly on the way confirmation steps are performed. Colonies can easily be misinterpreted as from coliform bacteria if not enough colonies of *A. hydrophila* are tested for oxidase reaction.

**Table 7B** presents the results outcome for *E. coli* confirmed after primary incubation at  $36\pm2$  °C. Mixture A and B contained both an *E. coli* strain while mixture C did not contain any. No differences appeared according to the method used for either mixture.

**Table 8** presents the results outcome for suspected thermotolerant coliform bacteria and confirmed *E. coli* from media incubated at 44/44.5 °C. For the analysis of suspected thermotolerant coliform bacteria the national methods were used as much as the method EN ISO 9308-1:2000. For *E. coli* the results are too few to draw any conclusion regarding differences (**Table 8B**). The results of suspected thermotolerant coliform bacteria reveal differences for all mixtures (**Table 8A**). In all cases the average value is on the lower side when Norwegian standard were used. Such trends cannot be seen for the other standard methods. The fact that Norwegian standard method gives often lower results is certainly due to an

**Table 9** Numbers of results, outliers excluded, with different method variants in the analysis of coliform bacteria (A) and *E. coli* (B) with membrane filtration

A. Coliform bacteria MF	Total no. of res.	Mixture					
		A		B		C	
		n	Mv <sup>1</sup>	n	Mv <sup>1</sup>	n	Mv <sup>1</sup>
<b>Medium</b>	<u>87</u>	<u>79</u>	<u>196</u>	<u>71</u>	<u>19</u>	<u>78</u>	<u>1253</u>
m-Endo Agar/Broth LES	60	58	197	54	18	57	1295
”LTTC Agar” <sup>2</sup>	18	16	205	13	19	16	1174
“Wrong information”	5	4	136	3	44	4	1013
Chromocult Agar	2	1	236	1	18	1	1182
Other	2	0	—	0	—	0	—
<b>Incubation temperature</b>	<u>87</u>	<u>77</u>	<u>196</u>	<u>71</u>	<u>19</u>	<u>78</u>	<u>1253</u>
35 °C	25	21	158	22	18	23	1251
36 °C	19	15	250	14	19	17	1283
37 °C	41	39	201	34	20	37	1239
Other	2	2	120	1	32	1	1300

**Table 9** *continued*

B. <i>Escherichia coli</i> MF	Total no. of res.	Mixture					
		A		B		C	
		n	Mv <sup>1</sup>	n	Mv <sup>1</sup>	n	Mv <sup>1</sup>
<b>Medium 35/36/37 °C<sup>3</sup></b>	<u>52</u>	<u>49</u>	<u>155</u>	<u>44</u>	<u>15</u>	<u>45</u>	<u>0</u>
m-Endo Agar/Broth LES	37	36	154	33	15	34	0
”LTTC Agar” <sup>2</sup>	12	11	152	9	15	9	0
<i>Wrong media vs. method</i> <sup>3</sup>	1	1	124	1	12	1	0
Chromocult Agar	2	1	236	1	18	1	0
Other	0	0	—	0	—	0	—
<b>Medium 44/44.5 °C<sup>4</sup></b>	<u>11</u>	<u>11</u>	<u>132</u>	<u>11</u>	<u>12</u>	<u>11</u>	<u>0</u>
m-FC Agar/Broth	7	7	129	7	10	7	0
”LTTC Agar” <sup>2</sup>	2	2	123	2	13	2	0
Other	2	2	152	2	21	2	0
<b>Incubation temperature</b>	<u>81</u>	<u>77</u>	<u>145</u>	<u>69</u>	<u>14</u>	<u>70</u>	<u>0</u>
From 35/36/37 °C	52	49	155	44	15	45	0
From 44/44.5 °C	12	12	130	12	12	12	0
From 36 or 44 °C	16	16	129	13	15	13	0
Unknown	1	0	—	0	—	0	—

1 Mean values calculated based on square root transformation

2 m-Lactose TTC (2,3,5-triphenyltetrazolium chloride) Agar + Tergitol 7 (heptadecylsulphate) according to EN ISO 9308-1:2000

3 Other media than m-Lactose TTC Agar given while referring to the standard XX.EN ISO 9308-1:2000

4 Results regarding confirmed *E. coli*; from method information for coliform bacteria

5 Results regarding confirmed *E. coli*; from method information for thermotolerant coliform bacteria – this is the reason why fewer results are present here for than for *E. coli* in total at 44/44.5 °C

incubation at 44.5 °C. The use of m-FC Agar medium and a temperature of 44.5 °C have especially shown to give low results, depending on the *E. coli* strain and perhaps also depending on the filter used. Inhibitors or selective agents have a bigger impact at high, and therefore more selective, temperature. Other factor of influence is the way confirmation steps are performed on suspected thermotolerant coliform bacteria. This can differ from one country to another and therefore be correlated to the used method.

**Table 9A** presents the results according to the media used for analyses. Small difference appears but not as evident as in **Table 7A**. This is certainly due to the fact that the differences are mainly caused by the confirmation steps (mixture B) or the interpretation of colonies (mixture A) and not directly by the medium used. The only result with Chromocult coliform Agar®, which is high and included *S. marcescens* as coliform bacterium, indicates that medium can play an important role in the outcome of the analysis.

The low results obtained at 35 °C compared to 36 and 37 °C are apparent and reflect the fact that it is mainly Swedish laboratories that used this temperature.

It is difficult to conclude anything for *E. coli* concerning the use of different media as the results are similar or too few to allow any comparison (**Table 9B**). Nevertheless it indicates that lower values are obtained for *E. coli* analysis after incubation at 44/44.5 °C compared to 36±2 °C.

### ***Clostridium perfringens* with membrane filtration methods (MF)**

#### **Various methods**

The analysis of *Clostridium perfringens* is performed in different ways in different countries and laboratories. This is due to the fact that no international standard is stated as reference method in the European drinking water directive (1). The parameters to be analysed according to the directive are spores and vegetative cells of *C. perfringens*. When this decision was taken, there was no international standard for water analyses. Therefore, one method was explicitly stated in the drinking water directive, namely the usage of m-CP Agar at 44 °C. This method includes a confirmatory step with ammonia vapour that makes *C. perfringens* colonies turning red.

Since many countries were unfamiliar with that method and as there was an ongoing standardisation work, there were desires to use the method in the standardisation process. An approval of using the most current standard draft was given by the group concerned under the EU commission. At that time, the method was available as a Committee Draft (CD), ISO/CD 6461-2:2002-12-20. Certain modifications were decided upon later at ISO standardisation meetings and communicated in the PT instructions of the National Food Agency. The last year's standardisation work has been started again in ISO and there is now a version in an even later stage, a Draft International Standard (DIS). This new standard proposition got the number ISO/DIS 14189 and should be finalized during 2013. In the main part it is similar to the CD version of 2002 with modifications but the confirmation step has been simplified: isolated, sub-cultured colonies should be tested for the acid phosphatase enzyme.

Another method that has been used is the older method for analysis of sulphite reducing anaerobic bacteria, EN ISO 26461-2:1993. It may have been used as it is, with or without heating of the sample, when it is comparable to the analysis of presumptive *C. perfringens*, or after a modification which makes it comparable to ISO/CD 6461-2:2002 by the introduction of a confirmation step for the identification of *C. perfringens*.

#### **Results**

In many cases it is unclear how exactly the methods were used. It is clear from **Table 10** that the mean values for the laboratories that reported presumptive results are lower in mixtures A and C when m-CP Agar was used, in comparison to the two other methods. This is in agreement with the results reported in spring 2008

**Table 10** Total number of method information answers and outcome of the results (outliers excluded) with different methods in analysis of *Clostridium perfringens* in mixture A and C

Method/"Standard"	Total no. of answ.	Mixture					
		A (pres. <sup>1</sup> )		A (conf. <sup>1</sup> )		C (pres. <sup>1</sup> )	
		n	Mv <sup>2</sup>	n	Mv <sup>2</sup>	n	Mv <sup>2</sup>
<b>With stated method, total</b>	<b>56</b>	<b>43</b>	<b>446</b>	<b>34</b>	<b>327</b>	<b>44</b>	<b>1028</b>
EN ISO 26461-2:1993 <sup>3</sup>	9	7	490	8	428	7	1487
ISO/CD 6461-2:2002 <sup>4</sup>	27	27	535	8	487	27	1347
EU directive (m-CP Agar) <sup>5</sup>	13	6	177	12	200	6	202
DS 2256 <sup>6</sup>	2	1	127	2	166	1	0
<i>Other</i>	5	2	363	4	375	3	467

1 pres. = presumptive *C. perfringens*; conf. = confirmed *C. perfringens*

2 Mean values calculated based on square root transformation for 100 ml sample

3 Water quality — Detection and enumeration of sulphite-reducing anaerobes (clostridia), Part 2: Method by membrane filtration (ISO 6461/2:1986)

4 Water quality — Detection and enumeration of *Clostridium perfringens*, Part 2: Method by MF

5 Council Directive 98/83/EC of 3 November 1998 (see reference 1)

6 Dansk Standard; Vandundersøgelse, Beståmmelse af *Clostridium perfringens*, 1 udg., Jan 1983

and 2011 (8, 11). Like in 2011, the same trend appears when looking at the confirmed results of mixture A. Even most other methods gave much lower results with the *C. perfringens* strain and the *C. bif fermentans* strain that were included in mixture A and C, respectively.

When using m-CP Agar, there are no presumptive results really, but they ought to be identical to the confirmed results. This seems to be true for mixture A, although results have not always been given by the same laboratory for both categories. Therefore it is possible that the “confirmed value” is higher than the “presumptive value”.

In total 23 out of 56 laboratories gave results for both presumptive and confirmed *C. perfringens* analyses. However, it is only partly the same laboratories that have reported both presumptive and confirmed results which implies that it is not totally possible to compare both analyses in mixture A.

**Table 10** shows clearly that the ways laboratories used EN ISO 26461-2:1993 and ISO/CD 6461-2:2002 they obtained approximately similar results.

**Table 11** presents the results obtained with different media independently of the method used. Here again, it is clear that lower values are obtained with the use of m-CP Agar for both mixture A and C.

All accepted analytical results were obtained after anaerobic incubation, mostly at 44 °C.

**Table 11** Total numbers of method information answers and outcome of the results (outliers excluded) with different substrates and different incubation temperatures in analysis of *Clostridium perfringens* in mixture A and C

Method variant	Total no. of answ.	Mixture					
		A (pres. <sup>1</sup> )		A (conf. <sup>1</sup> )		C (pres. <sup>1</sup> )	
		n	Mv <sup>2</sup>	n	Mv <sup>2</sup>	n	Mv <sup>2</sup>
<b>Medium</b>	<b>56</b>	<b>43</b>	<b>446</b>	<b>34</b>	<b>327</b>	<b>44</b>	<b>1028</b>
“PAB/TSC Agar” 44 °C <sup>3</sup>	37	34	545	17	507	34	1520
“SFP Agar” <sup>4</sup>	1	0	—	0	—	1	0
m-CP Agar <sup>5</sup>	15	7	189	14	203	9	149
Iron Sulphate Agar <sup>6</sup>	3	2	85	3	121	2	0
<i>Other</i>	0	0	—	0	—	0	—

1 pres. = presumptive *C. perfringens*; conf. = confirmed *C. perfringens*

2 Mean values calculated based on square root transformation

3 Perfringens Agar base / Tryptose Sulphite Cycloserine Agar; was here used with D-cycloserine.

4 SFP Agar contains Polymyxine & Kanamycin.

5 m-CP Agar contains D-cycloserine & Polymyxine.

6 No specific antibiotic is included in Iron Sulphate Agar.

## Moulds and yeasts in water (MF)

### Various methods

Out of 40 laboratories that gave method information, 34 used the Swedish standard SS 028192. Besides Sweden, this standard is used in Denmark, and Finland and Norway, but under the national designations SFS 5507 and NS 4716, respectively. Furthermore, 6 laboratories used their own method, food methods and one method from Standard Methods of Water and Wastewater (6).

### Results

As in previous rounds when method information regarding the fungal analyses was collected, for example 2005 (7), many different media were used (**Table 12**). Even though most are suitable for this analysis, all are not.

DG 18 is a medium with low water activity and is developed for xerophilic fungi, i.e. fungi that grow on dry media. This medium is commonly used for food analyses. Results derived from DG 18 can therefore be expected to be somewhat lower than for most other media used when fungi that thrive in aquatic environments are included. Only one laboratory used this medium.

Malt Extract Agar is a common medium for micro-fungi analysis and is less inhibiting for rapidly growing fungi, such as *Rhizopus sp.* and *Mucor sp.*, than the other used media. The inhibition level of the various media towards bacterial growth depends on whether suitable antibiotics are added. Some media contain Rose Bengal, a pigment with growth inhibiting properties and antibacterial

chemicals such as chlortetracycline, chloramphenicol or oxytetracycline. Moreover, DRBC Agar and DG 18 contain low concentration of dichloran which, in addition to Rose Bengal, restrains the growth of rapidly growing fungi, and hence, facilitates the interpretation of other fungi.

**Table 12** *Colony count on different agar media for micro fungi analysis of mixtures A, B and C, outliers excluded. Recommended supplements in the different media are indicated within parentheses in the notes.*

Medium	Cooke <sup>1</sup>	RBC <sup>2</sup>	DRBC <sup>3</sup>	OGYE <sup>4</sup>	ME <sup>5</sup>	DG <sup>6</sup>	18	Other <sup>7</sup>
<b><u>Mould</u></b>								
<b>Mixture A</b>								
No. of lab.	12	10	8	2	3	1	1	
Min	8	4	7	7	5	11	5	
Median	<b>12</b>	<b>12</b>	<b>12</b>	<b>11</b>	<b>13</b>	<b>11</b>	<b>5</b>	
Max	16	16	14	16	13	11	5	
<b>Mixture B</b>								
No. of lab.	12	9	8	3	3	1	0	
Min	100	36	73	20	120	530		
Median	<b>456</b>	<b>300</b>	<b>169</b>	<b>100</b>	<b>180</b>	<b>530</b>		
Max	810	670	523	300	1000	530		
<b><u>Yeast</u></b>								
<b>Mixture B</b>								
No. of lab.	5	5	6	1	2	0	1	
Min	391	482	360	465	440		580	
Median	<b>514</b>	<b>520</b>	<b>495</b>	<b>465</b>	<b>503</b>		<b>580</b>	
Max	600	590	700	465	570		580	
<b>Mixture C</b>								
No. of lab.	12	9	10	3	3	1	1	
Min	600	626	740	600	727	800	830	
Median	<b>746</b>	<b>760</b>	<b>835</b>	<b>768</b>	<b>780</b>	<b>800</b>	<b>830</b>	
Max	1132	1027	1100	970	800	800	830	

1 Cooke Rose bengal Agar (chlortetracycline)

2 Rose bengal Agar (chloramphenicol)

3 Dichloran Rose bengal Agar (chloramphenicol)

4 Glucose Yeast extract Agar (oxytetracycline)

5 Malt extract Agar

6 Dichloran Glycerol (18%) Agar (chloramphenicol)

7 Sabouraud Agar (no supplement)

Based on the national standards prescriptions, more supplements were added to the different media than the antibiotic recommended by manufacturers (see notes for **Table 12**). For example, Swedish laboratories use often both chlortetracycline and chloramphenicol according to the standard SS 028192.

The results from the various media as stated by the laboratories or after our interpretation based on product designation, regardless of which antibiotic are really used, is clear from **Table 12**. No outliers are included. The results are stated as median, lowest and highest values. For moulds, no differences appear for mixture A, but the results are lower for mixture B when DRBC, OGYE and ME Agar were used, compared to Cooke's Rose Bengal Agar and RBC. The used of these medium did not lead to such difference for yeast analysis of mixture B or C. The total median values for the respective mixture is given in **Annex A**.

**Table 13** Number of method answers (n) and average values of the results (Mv), outliers excluded, for mould and yeast analyses with different methods variations.

Methods variations	Tot	Moulds A		Moulds B		Yeast B		Yeast C	
	n	n	Mv <sup>1</sup>						
<b>Growth inhibitors</b>									
Rose bengal	12	12	10	10	234	8	515	11	786
Dichloran	6	6	11	4	230	4	495	6	845
<b>Antibiotics</b>									
Only chlortetracycline	1	1	14	1	460	0	–	1	830
Only chloramphenicol	10	8	12	8	329	5	493	10	814
Chlortetra. + chloramph.	20	20	11	19	315	9	495	19	843
Oxytetracycline	4	3	10	4	109	2	462	4	781
Other	4	4	8	3	515	2	575	4	789
<b>Incubation temperature</b>									
<24 °C	6	5	9	3	286	3	552	6	861
24-26 °C	34	32	12	33	291	17	512	33	809
>26 °C	0	0	–	0	–	0	–	0	–
Unknown	0	0	–	0	–	0	–	0	–
<b>Incubation time</b>									
<5 days	0	0	–	0	–	0	–	0	–
5 days	5	4	9	3	223	3	511	5	812
6 days	0	0	–	0	–	0	–	0	–
7 days	35	33	11	33	298	17	519	34	817
>7 days	0	0	–	0	–	0	–	0	–
Unknown	0	0	–	0	–	0	–	0	–
<b>All incoming results</b>	<b>40</b>	<b>37</b>	<b>11</b>	<b>36</b>	<b>291</b>	<b>20</b>	<b>518</b>	<b>39</b>	<b>817</b>

The results regarding various circumstances listed in **Table 13** show clearly that the laboratories that used oxytetracycline, that is OGYE medium, got lower values for mould analysis of mixture B. Such trend did not appear for any of the other analyses.

Several laboratories added both chloramphenicol and chlortetracycline to the medium, often Cooke's Rose Bengal, according to the standard SS 028192. However no evident differences could be noticed in **Table 13** when only one of the antibiotic was added.

We suspect that many laboratories using media already containing Rose Bengal are not aware of it, which could explain the low number of laboratories that reported the use of this supplement.

Globally, the temperature and time of incubation did not strongly influence the results obtained. Looking at the mould analyses, even though few results were reported for 5 days of incubation, it seems there is a trend for the values to be slightly lower than for 7 days of incubation.

## The outcome of deviating results – assessment

The results of all laboratories are listed in **Annex A**. A summary of the results of each laboratory – false results excluded – is illustrated by a box plot based on their z-scores (**Figure 2**). The smaller, and the more centred around zero the box of a laboratory is, the closer its results are to the general mean values calculated for all laboratory results.

The laboratories are not grouped or ranked based on their results. However, the assessment aims to clearly give information regarding the number of false results and outliers which are presented below the box plots. These results are also highlighted in **Annex A**, where also the minimum and maximum accepted values for each analysis are stated in the summarizing rows at the end.

In cases where it is obvious, it is also stated if a laboratory has mixed up the analytical results. If mixtures have been mixed up, it is shown by crossing of their sample numbers in **Annex A**. No laboratory seems to have mixed up the results from individual analyses this time. Neither can it be suspected that laboratories have missed to give their results for the volumes asked for, namely 100 ml in all analyses except for culturable microorganisms where 1 ml is appropriate.

Z-values listed in **Annex B** are the base for the box plots but they are not commented or evaluated. They can be used by laboratories in their follow-up process.

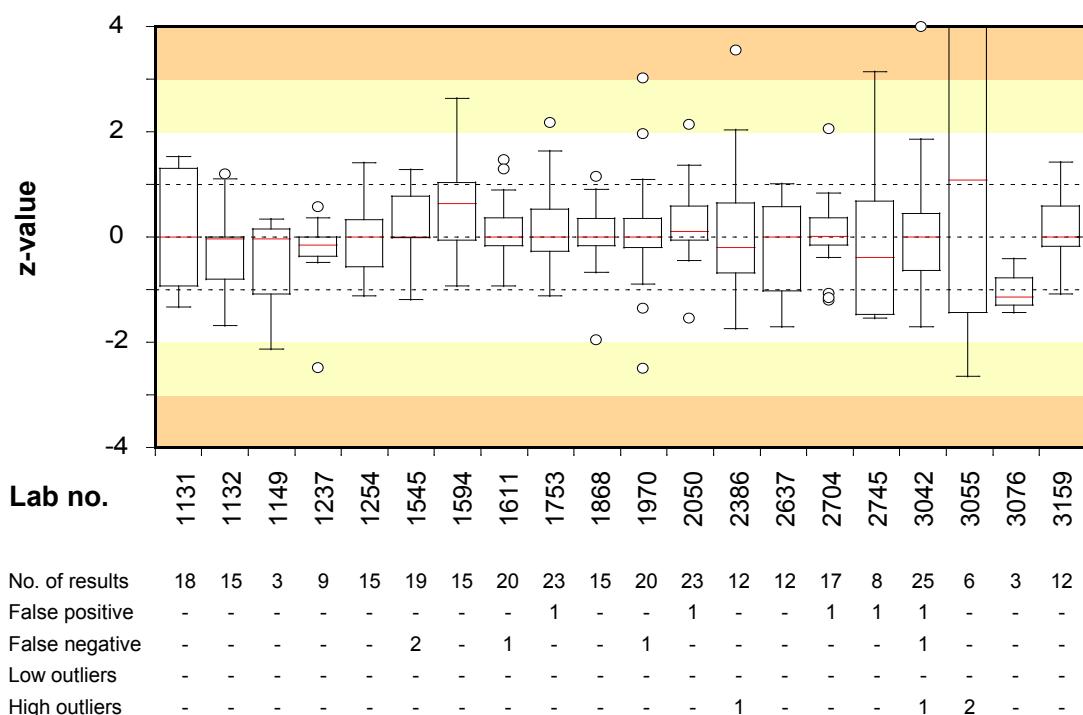
Description of the result processing and recommendations on follow-up work are given in the scheme protocol (3). A PDF file of that document is available on the website [www.slv.se/absint](http://www.slv.se/absint).

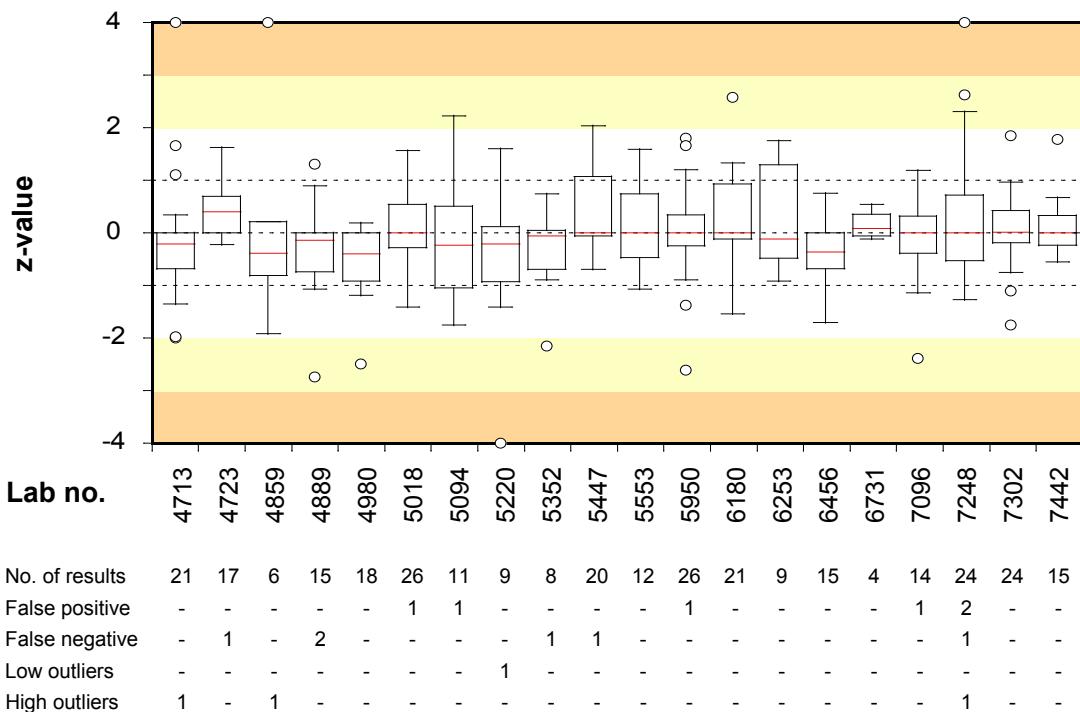
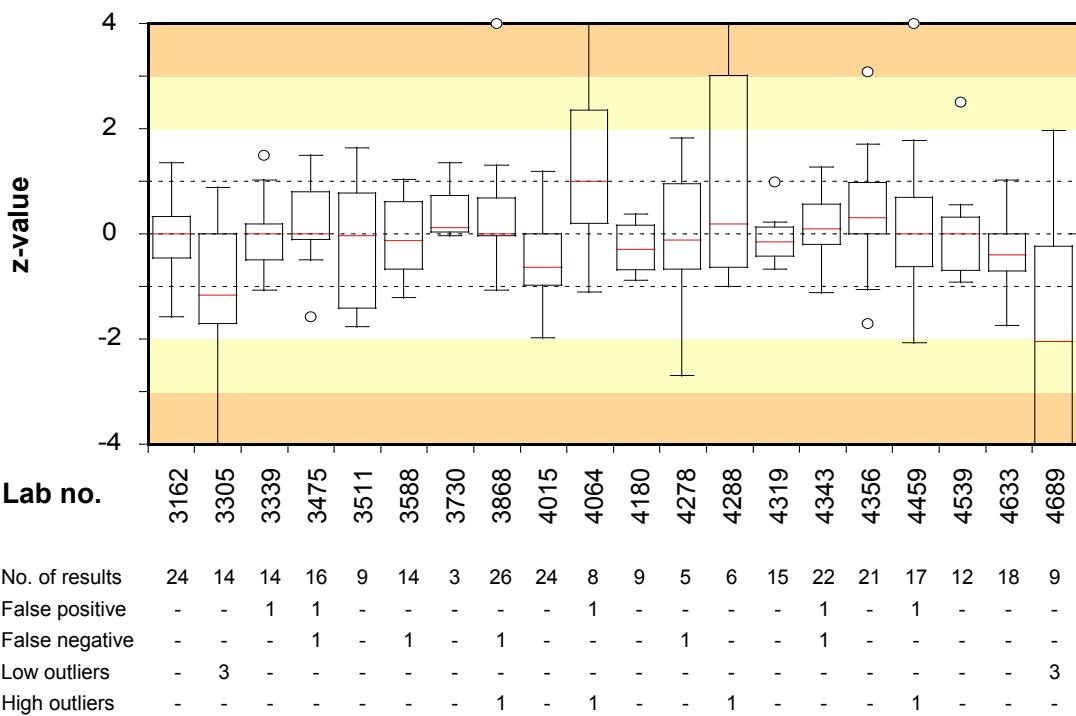


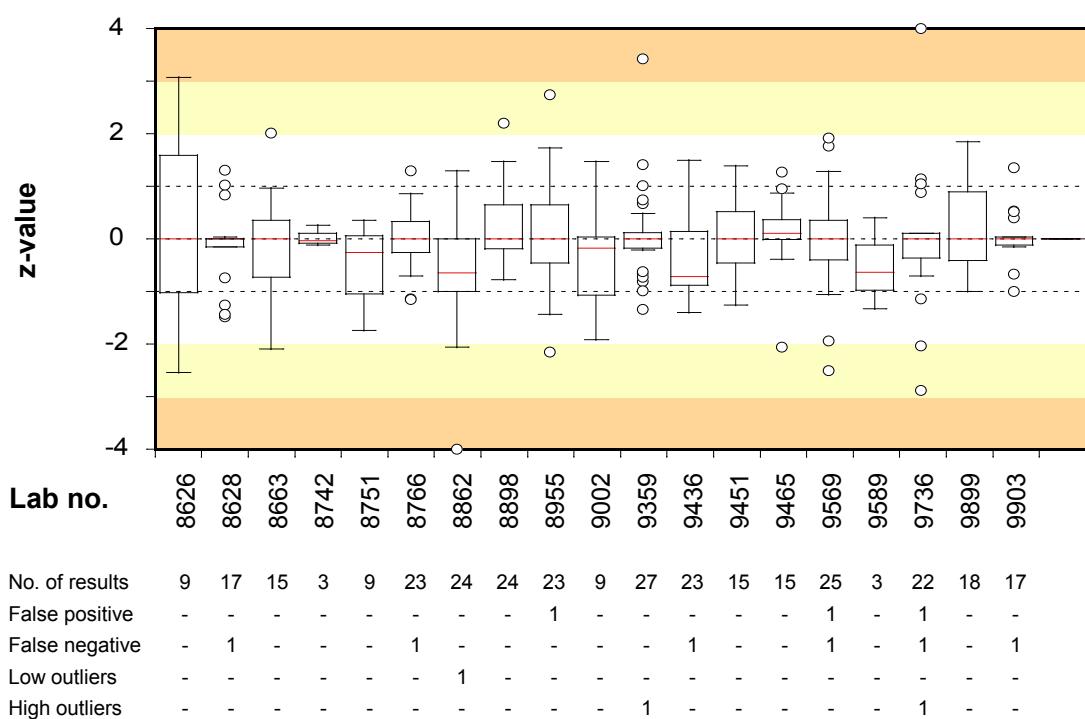
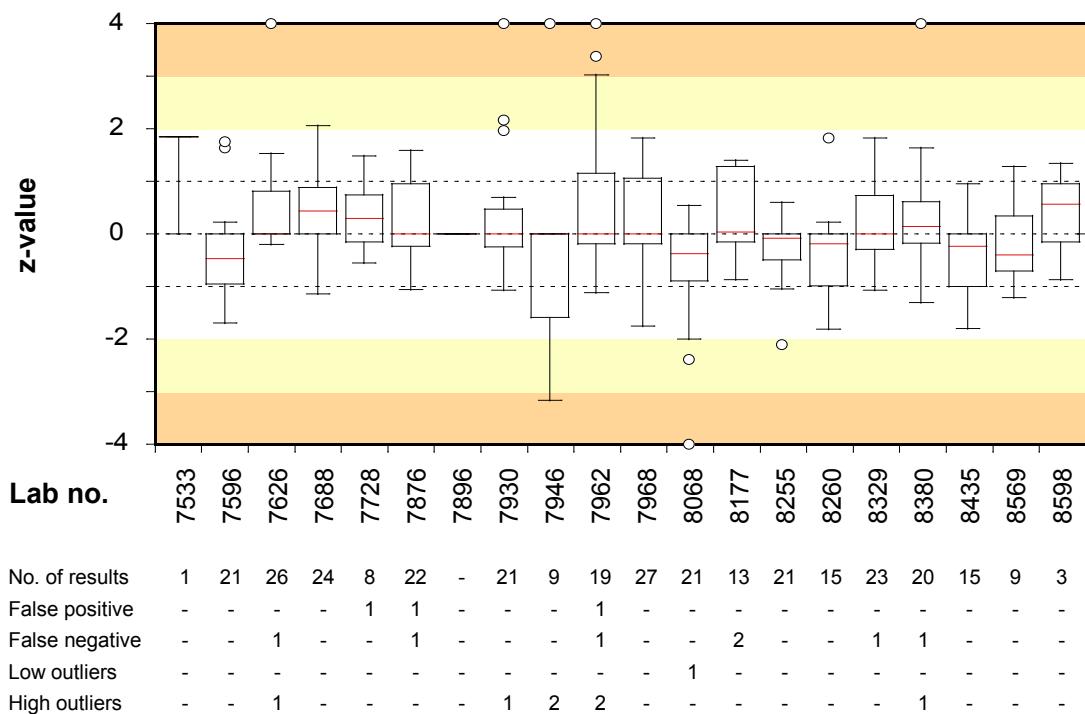
**Figure 2** Box plots and number of deviating values for each participating laboratory. The square root transformed results of a laboratory is converted into standardised values (*z*-value) to be able to compare the different analyses.

- Standardised values are calculated from the formula  $z = (x - mv) / s$
- Standardised values  $> +4$  and  $< -4$  have in the plots received the values  $+4$  and  $-4$ , respectively.
- False results do not generate *z* values and are not included in 'No. of results'. False positive results cannot be illustrated in the box plots. The no. of false positives and false negatives are clear from the table beneath the plots.
- The outliers are included in the plots after recalculation to standardised values with the same *s* values as the rest of the results. The nos. is clear from the table.
- The horizontal line in each box indicates the median of the laboratory.
- The two box area parts embrace 25% of the results above and below the median, respectively. The lines reaching out from the box and/or the circles embrace the remaining 50% of the results, false results excluded.
- A circle is created when a result is highly deviating\* from the rest.
- The background is decorated with lines and shaded fields to indicate ranges in order to simplify localisation 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  $> [\text{largest value of the box} + 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$







## References

1. 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 (*there are national translations*).
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. Anonymous 2007. Proficiency Testing Schemes, Protocol, Microbiology, Drinking water & Food. National Food Administration, Uppsala, 26 p.
4. Kelly, K. 1990. Outlier detection in collaborative studies. J. Assoc. Off. Chem. 73:58-64.
5. Niemi, R. M., Mentu, J., Siionen, A., Niemelä, S. I. 2003 Confirmation of *Escherichia coli* and its distinction from *Klebsiella* species by gas and indole formation at 44 and 44,5 °C. Journal of Applied Microbiology 95, 1242-1249.
6. Standard Methods for the Examination of Water and Wastewater, <http://www.standardmethods.org/>
7. Šlapokas, T., Gunnarsson. 2005. Proficiency Testing, Drinking water microbiology, 2005:2 (September). National Food Administration report no. 29-2005, Uppsala, 39 p.
8. Šlapokas, T., Gunnarsson, C., Jentzen, A. 2008. Proficiency Testing, Drinking water microbiology, 2008:1 (March). National Food Administration report no. 13-2008, Uppsala, 37 p.
9. Šlapokas, T., Jentzen, A. 2008. Proficiency Testing, Drinking water microbiology, 2008:2 (September). National Food Administration report no. 27-2008, Uppsala, 39 p.
10. Šlapokas, T., Lantz, C., Olsson M. 2010. Proficiency Testing, Drinking water microbiology, 2010:1 (March). National Food Administration report no. 9-2010, Uppsala, 40 p.
11. Šlapokas, T., Lantz, C., Lindqvist, M. 2011. Proficiency Testing, Drinking water microbiology, 2011:1 (March). National Food Administration report no. 13-2011, Uppsala, 47 p.

**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. This is also valid for results in **shaded columns**. **Empty hatched fields** indicate that the result has been deleted due to misunderstanding of instructions or use of improper method. A **hyphen** indicate that no result has been reported. **Figures written in bold in yellow fields** indicate outliers, false positive and false negative results. **Underlined zero values** indicate results characterized as 'False negative ?'. **Crossed out sample numbers** on a line indicate that the samples probably are mixed up. False positive and false negative values are excluded, as well as other outliers,

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	3 2 1	110	200	1600	110	7	1600	-	-	-	110	7	0	387	21	1350	162	21	0
1132	3 2 1	-	-	-	-	-	-	87	6	245	87	6	0	326	19,5	1199	133	19,5	0
1149	3 1 2	150	110	1170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	1 3 2	-	-	-	160	-	1400	-	-	-	160	-	<1	165	14	>200	145	14	<1
1254	3 2 1	170	18	990	170	13	990	150	11	310	150	11	0	-	-	-	-	-	-
1545	3 1 2	160	160	1500	160	0	1500	160	0	430	160	0	-	-	-	-	-	-	-
1594	2 1 3	570	156	1200	570	10	1200	120	16	450	230	10	0	523	19	1540	181	19	0
1611	1 2 3	380	190	1200	380	10	1200	166	14	480	160	10	0	451	15	1323	175	15	0
1753	3 2 1	168	18	1464	168	18	1464	-	-	-	168	18	0	284	22	1279	152	21	0
1868	1 3 2	158	173	1240	158	17	1240	-	-	-	158	17	0	499	16	1567	199	16	0
1970	2 3 1	190	140	1300	190	70	1300	150	12	380	150	12	0	-	-	-	-	-	-
2050	2 3 1	-	-	-	162	17	1436	-	-	-	162	17	0	500	15	1939	168	15	0
2386	3 1 2	130	140	1600	130	50	1600	-	-	-	114	50	0	-	-	-	-	-	-
2637	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	280	17	1600	110	17	<1
2704	3 1 2	-	-	-	180	15	1000	-	-	-	180	15	510	429	16	1441	124	16	<1
2745	1 3 2	180	6	900	180	6	900	180	6	900	180	6	900	-	-	-	-	-	-
3042	3 1 2	18	3	11	440	29	1100	18	2	11	160	15	440	2200	12	1440	110	11	<1
3055	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	720	380	720	-	-	-
3076	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3159	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	288	17,8	1298	150	16,4	<1
3162	2 1 3	165	170	1390	165	20	1390	-	-	-	165	20	0	387	16	1112	206	16	0
3305	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	25	10	95	10	9	0
3339	3 1 2	210	138	1000	210	14	1000	-	-	-	210	14	0	-	-	-	-	-	-
3475	2 1 3	-	-	-	155	14	891	-	-	-	155	14	0	435	19	1300	196	19	<1
3511	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	504	8,7	1652	118	8,7	<1
3588	2 3 1	170	150	1409	170	14	1409	102	10	165	102	10	0	-	-	-	-	-	-
3730	2 1 3	200	200	1600	-	-	-	200	12	460	-	-	-	-	-	-	-	-	-
3868	1 3 2	170	110	1600	170	20	1600	95	11	450	170	20	0	450	18	1450	140	18	0
4015	3 1 2	119	21	1104	119	11	1104	85	6	322	119	8	0	205	10	1299	133	10	0
4064	2 3 1	-	80	-	434	67	1325	-	-	-	217	56	442	-	-	-	-	-	-
4180	2 3 1	-	-	-	120	16	1350	-	-	-	120	16	0	-	-	-	-	-	-
4278	2 1 3	-	-	-	15	46	0	-	-	-	-	-	-	-	-	-	-	-	-
4288	3 2 1	-	-	-	105	110	1100	-	-	-	-	-	-	-	-	-	-	-	-
4319	3 2 1	134	125	1298	134	15	1298	-	-	-	128	15	0	355	14	1378	167	14	0
4343	1 3 2	182	136	1591	182	17	1591	-	-	-	182	17	0	285	16	1120	147	16	0
4356	2 3 1	180	146	1510	180	44	1510	-	-	-	180	44	0	290	18	1700	170	18	0
4459	1 2 3	-	-	-	80	102	791	-	-	-	80	10	475	520	18	1300	222	16	<1
4539	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	306	16	1091	178	15	0
4633	1 3 2	-	-	-	154	21	1309	133	12	382	133	12	0	341	12	1201	143	12	0
4689	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	49	7,8	350	49	7,8	0
4713	3 1 2	410	73	1200	410	13	1200	-	-	-	120	13	0	320	8	1400	140	8	0
4723	1 2 3	173	173	1200	173	28	1200	101	11	282	173	28	0	-	-	-	-	-	-
4859	3 2 1	-	-	-	120	96	1070	90	71	410	80	16	0	-	-	-	-	-	-
4889	3 1 2	-	-	-	140	<1	1600	-	-	-	140	<1	<1	520	6	1300	160	6	<1
4980	1 2 3	121	21	710	121	21	710	109	10	400	109	10	0	429	13,7	1091	144,5	13,7	<1
5018	2 1 3	120	65	1200	120	26	1200	-	-	-	120	26	0	613	19	1203	167	19	0
5094	2 3 1	500	52	1100	500	52	1100	108	8	500	108	8	0	-	-	-	-	-	-
5220	1 3 2	-	-	-	400	10	1200	-	-	-	150	10	0	-	-	-	-	-	-
5352	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5447	1 3 2	-	-	-	236	18	1182	-	-	-	236	18	0	-	-	-	-	-	-
5553	3 2 1	-	-	-	136	26	1000	-	-	-	124	12	0	-	-	-	-	-	-
5950	1 2 3	140	164	1400	140	19	1400	120	9	420	170	19	0	649	15	1789	119	15	0
6180	3 1 2	560	155	900	560	15	900	160	7	520	200	15	0	531	15	1110	178	15	0
6253	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	305	22	1680	150	22	0
6456	1 2 3	-	-	-	115	14	1250	-	-	-	110	12	0	450	13	1021	185	13	0
6731	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	>1	>1	>1	>1	>1	0
7096	2 1 3	157	14	1220	157	14	1220	133	9	339	157	14	0	-	-	-	-	-	-
7248	1 2 3	137	110	1318	137	55	1318	100	147	0	100	147	0	687,4	14,73	1534	254,9	14,73	0
7302	3 2 1	227	155	1073	227	20	1073	-	-	-	227	20	0	461	14	1354	173	14	0
7442	1 2 3	150	149	1200	150	18	1200	-	-	-	150	18	0	387	16	1181	182	16	0
7533	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	>23	23	>23	-	-	-
7596	3 2 1	220	121	1140	220	11	1140	113	8	410	113	8	0	276	22	1200	131	22	0
7626	1 3 2	250	99	1250	250	19	1250	139	19	0	139	19	0	495	21	1597	160	21	0
7688	1 3 2	-	-	-	460	20	1800	120	20	700	120	20	0	488	17	1414	179	17	0
7728	1 3 2	-	-	-	178	19	1650	-	-	-	178	19	1150	-	-	-	-	-	-
7876	3 1 2	150	180	1280	150	19	1280	90	14	520	150	8	<1	369	20	1076	170	20	<1
7896	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7930	2 1 3	490	9	1330	490	9	1330	-	-	-	110	9	0	487	14	1421	487	14	0
Mean					196	19	1253				145	14	0	406	15	1320	160	15	0
CV (%)					27	30	10				13	24	-	15	13	10	10	13	-

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-scores 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.  $z = (x - \text{mv}) / s$ .

Presumptive C. <i>perfringens</i> (MF)			C. <i>perfringens</i> (MF)			Mould (MF)			Yeast (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	
890	0	0	-	-	-	-	-	-	-	-	-	20	76	18	1131
-	-	-	79	0	0	-	-	-	-	-	-	12	82	15	1132
-	-	-	-	-	-	-	-	-	-	-	-	12	55	17	1149
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1237
-	-	-	-	-	-	14	86	0	0	640	880	15	84	13	1254
720	0	3000	720	0	0	14	73	0	0	500	840	12	72	20	1545
-	-	-	-	-	-	-	-	-	-	-	-	27	83	15	1594
-	-	-	-	-	-	16	550	0	0	0	755	9	86	15	1611
596	0	21	-	-	-	12	150	0	4	514	1132	9	77	18	1753
-	-	-	-	-	-	-	-	-	-	-	-	9	57	15	1868
470	0	4200	470	0	0	7	0	0	0	490	1100	8	51	19	1970
370	0	2627	-	-	-	13	36	0	50	482	1009	15	92	15	2050
-	-	-	200	0	0	-	-	-	-	-	-	9	76	9	2386
-	-	-	60	<1	<1	-	-	-	-	-	-	8	91	13	2637
-	-	-	330	0	0	-	-	-	-	-	-	25	89	11	2704
-	-	-	-	-	-	-	-	-	-	-	-	15	130	13	2745
127	0	0	165	0	0	14	460	0	0	0	830	20	75	16	3042
-	-	-	-	-	-	-	-	-	-	-	-	60	80	10	3055
-	-	-	300	0	0	-	-	-	-	-	-	7	76	10	3076
320	0	640	-	-	-	13	100	0	0	480	620	10	72	14	3162
170	0	0	0	0	0	-	-	-	-	-	-	5	94	19	3305
490	18	60	490	0	0	-	-	-	-	-	-	18	84	13	3339
-	-	-	0	0	345	-	-	-	-	-	-	21	90	15	3475
-	-	-	-	-	-	10	0	0	0	590	960	9	91	12	3511
-	-	-	-	-	-	-	-	-	-	-	-	12	101	16	3588
520	0	0	520	0	0	11	0	0	0	1000	810	16	80	15	3868
836	0	4250	-	-	-	9	81	0	0	518	626	9	86	18	4015
-	-	-	-	-	-	-	-	-	-	-	-	14	67	16	4064
-	-	-	-	-	-	-	-	-	-	-	-	13	77	12	4180
-	-	-	-	-	-	-	-	-	-	-	-	9	95	15	4278
-	-	-	-	-	-	-	-	-	-	-	-	11	90	31	4288
-	-	-	-	-	-	-	-	-	-	-	-	9	76	20	4319
685	0	541	-	-	-	13	564	0	7	0	773	17	84	18	4343
260	0	5000	230	0	0	-	-	-	-	-	-	5	93	20	4356
545	<1	2800	-	-	-	-	-	-	-	-	-	11	80	13	4459
-	-	-	167	0	0	-	-	-	-	-	-	14	73	28	4539
776	0	0	-	-	-	7	100	0	0	920	970	14	79	14	4633
-	-	-	-	-	-	11	486	0	0	0	910	17	96	15	4713
564	0	5545	-	-	-	-	-	-	-	-	-	-	-	-	4723
-	<1	<1	-	-	-	-	-	-	-	-	-	8	74	14	4859
-	-	-	101	0	0	-	-	-	-	-	-	11	66	13	4980
530	0	1770	530	0	0	13	120	0	171	440	780	6	78	22	5018
-	-	-	-	-	-	300	-	198	-	600	-	11	67	20	5094
-	-	-	-	-	-	5	<1	<1	<1	580	830	8	75	15	5220
718	0	6800	718	0	0	13	1000	0	0	0	727	11	101	15	5352
-	-	-	600	0	0	-	-	-	-	-	-	21	80	23	5447
380	0	4600	380	0	0	4	200	0	12	520	760	8	82	17	5553
740	0	4100	740	0	0	-	-	-	-	-	-	11	75	15	5950
-	-	-	-	-	-	-	-	-	-	-	-	9	75	15	6180
-	-	-	-	-	-	-	-	-	-	-	-	5	89	15	6253
-	-	-	-	-	-	-	-	-	-	-	-	13	89	15	6456
-	-	-	-	-	-	-	-	-	-	-	-	7	96	7	6731
750	0	1740	690	0	740	-	-	-	-	-	-	10	78	24	7096
255	0	31	255	0	31	9	582	0	92	0	905	10	69	16	7248
636	0	3200	-	-	-	10	300	0	0	600	600	9	67	17	7302
-	-	-	-	-	-	-	-	-	-	-	-	10	78	24	7442
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7533
102	0	0	102	0	0	-	-	-	-	-	-	12	71	15	7596
643	0	990	643	0	0	235	523	0	0	0	791	12	97	16	7626
-	-	-	690	0	0	14	140	0	0	700	950	7	111	15	7688
-	-	-	-	-	-	-	-	-	-	-	-	16	74	15	7728
570	<1	90	-	-	-	15	810	<1	20	<1	1000	11	95	15	7876
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7896
270	0	0	270	0	0	-	-	-	-	-	-	16	88	25	7930
446	0	1028	327	0	0	11	291	0	0	518	817	12	81	16	Mean
31	-	93	38	-	-	15	42	-	8	8	8	21	8	14	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
7946	2 1 3	140	139	180	-	-	-	40	100	180	-	-	-	-	-	-	-	-	-
7962	3 2 1	160	196	1900	160	78	1900	140	7	410	160	78	400	435	14	1046	196	14	0
7968	2 3 1	435	116	1550	435	10	1550	100	10	185	195	10	0	477	14	1725	175	14	0
8068	1 3 2	147	34	1000	147	12	1000	100	9	810	147	12	0	308	8	770	50	8	0
8177	1 3 2	200	140	1600	200	0	1600	-	-	-	200	0	0	590	14	1700	150	14	0
8255	1 2 3	-	-	-	170	14	1400	160	12	570	170	14	0	390	11	1300	160	11	0
8260	1 3 2	177	116	939	177	22	939	138	30	425	83	30	<1	-	-	-	-	-	-
8329	3 2 1	139	131	1495	139	13	1495	158	15	550	131	13	0	435	15	1203	214	15	0
8380	1 2 3	160	98	1400	160	27	1400	160	27	1400	160	27	<1	330	16	1000	160	16	<1
8435	1 2 3	140	10	1500	140	10	1500	89	10	400	140	10	0	-	-	-	-	-	-
8569	3 2 1	130	162	970	130	12	970	130	9	380	130	9	0	-	-	-	-	-	-
8598	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8626	3 2 1	170	140	800	119	42	700	170	140	800	68	42	0	-	-	-	-	-	-
8628	2 3 1	-	-	-	180	18	1600	93	7	530	93	7	0	-	-	-	-	-	-
8663	2 1 3	120	160	1300	120	32	1300	90	9	340	120	32	0	480	12	830	170	12	0
8742	1 3 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8751	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	435	11	1414	162	11	<1
8766	2 3 1	145	155	1200	145	16	1200	145	16	482	145	16	<1	345	16	1553	155	16	<1
8862	1 3 2	180	21	1291	180	11	1291	-	-	-	180	11	0	317	9	1130	134	9	0
8898	1 2 3	495	99	1369	495	13	1369	-	-	-	209	13	0	478	14	1727	196	14	0
8955	3 1 2	200	40	1100	200	40	1100	-	-	-	200	40	0	490	13	1600	220	13	<1
9002	3 2 1	-	-	-	200	40	1000	-	-	-	80	8	0	-	-	-	-	-	-
9359	3 1 2	120	22	1400	120	18	1200	-	-	-	150	18	0	390	15	1300	120	15	0
9436	3 2 1	118	105	964	118	9	964	118	5	300	118	9	<1	261	16	980	138	16	<1
9451	2 1 3	420	30	1400	140	30	1400	113	7	490	113	7	0	-	-	-	-	-	-
9465	3 2 1	352	111	1227	352	15	1227	78	0	18	145	15	0	517	16	1414	192	16	<1
9569	2 1 3	350	116	1730	350	9	1730	120	5	470	130	9	<1	365	15	1414	138	14	<1
9589	2 3 1	-	-	-	240	7	1100	-	-	-	-	-	-	-	-	-	-	-	-
9736	1 3 2	182	25	1171	182	12	1171	-	-	-	182	12	0	201	15	1203	81	15	0
9899	1 2 3	227	131	1486	227	11	1486	-	-	-	227	11	0	-	-	-	-	-	-
9903	3 1 2	198	208	1216	198	18	1216	140	14	825	140	14	0	-	-	-	-	-	-

n	60	61	60	79	78	79	44	44	44	77	76	77	60	61	59	59	59	60
Min	18	3	11	15	0	0	18	0	0	68	0	0	25	6	95	10	6	0
Max	570	208	1900	570	110	1900	200	147	1400	236	147	1150	2200	380	1939	487	22	0
Median	170	121	1245	170	17	1245	120	10	415	150	14	0	429	15	1300	160	15	0
Mean				196	19	1253				145	14	0	406	15	1320	160	15	0
CV (%)				27	30	10				13	24	-	15	13	10	10	13	-
False positive	-	-	-	0	0	0	-	-	-	0	0	7	0	0	0	0	0	0
False negative	-	-	-	0	3	1	-	-	-	0	3	0	0	0	0	0	0	0
Outliers, low	-	-	-	0	0	0	-	-	-	0	0	0	2	0	2	3	0	0
Outliers, high	-	-	-	0	4	0	-	-	-	0	4	0	1	1	0	1	0	0
Low limit OK	18	3	11	15	6	700	18	0	0	68	6	0	165	6	720	81	6	0
High limit OK	570	208	1900	570	70	1900	200	147	1400	236	44	0	720	23	1939	255	22	0

mv ( $\sqrt{Mean}$ )				13,992	4,388	35,396				12,055	3,802	0,000	20,155	3,851	36,335	12,647	3,816	0,000
s ( $CV \cdot mv / 100$ )				3,753	1,313	3,516				1,625	0,919	0,000	2,948	0,512	3,599	1,264	0,499	0,000
x ( $\sqrt{Result}$ )																		
z ( $(x-mv)/s$ )																		

Presumptive <i>C. perfringens</i> (MF)			<i>C. perfringens</i> (MF)			Mould (MF)			Yeast (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	
52	0	0	52	0	0	-	-	-	-	-	-	44	44	106	7946
-	-	-	-	-	-	11	670	0	0	0	700	11	128	15	7962
760	0	3800	760	0	0	16	20	0	0	465	768	22	81	12	7968
240	0	1250	240	0	0	-	-	-	-	-	-	8	89	15	8068
-	-	-	-	-	-	-	-	-	-	-	-	13	70	20	8177
-	-	-	-	-	-	9	200	0	0	360	740	11	82	16	8255
365	<1	<1	365	<1	<1	-	-	-	-	-	-	8	65	12	8260
1091	0	0	-	-	-	13	695	0	0	0	715	22	83	23	8329
-	-	-	-	-	-	30	520	<1	<1	<1	900	22	71	14	8380
88	0	0	88	0	0	-	-	-	-	-	-	11	61	18	8435
-	-	-	-	-	-	-	-	-	-	-	-	18	100	17	8569
-	-	-	-	-	-	-	-	-	-	-	-	20	70	18	8598
-	-	-	-	-	-	-	-	-	-	-	-	33	68	19	8626
-	-	-	170	0	0	11	530	0	0	0	800	18	82	10	8628
-	-	-	-	-	-	-	-	-	-	-	-	11	87	12	8663
-	-	-	-	-	-	-	-	-	-	-	-	12	85	15	8742
-	-	-	-	-	-	-	-	-	-	-	-	7	78	9	8751
273	<1	5000	-	-	-	14	427	<1	<1	<1	802	7	88	11	8766
773	0	0	-	-	-	16	110	0	0	391	709	13	5	8	8862
668	0	273	-	-	-	11	132	0	0	545	720	12	89	21	8898
-	-	-	455	0	0	5	180	1	0	570	800	9	85	10	8955
-	-	-	-	-	-	-	-	-	-	-	-	6	79	18	9002
770	0	2700	770	0	0	9	100	0	0	460	810	36	92	13	9359
255	<1	5900	-	-	-	8	591	<1	<1	<1	709	21	87	12	9436
300	0	470	300	0	0	-	-	-	-	-	-	17	88	22	9451
-	-	-	-	-	-	-	-	-	-	-	-	13	83	8	9465
23	<1	800	23	<1	800	14	610	<1	<1	<1	690	24	100	16	9569
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9589
413	0	4364	-	-	-	15	300	0	37	0	1500	7	83	15	9736
423	0	6909	-	-	-	9	99	0	0	568	1027	15	76	22	9899
563	0	6	-	-	-	13	431	0	0	0	1006	9	82	15	9903

43	44	44	35	36	36	39	40	39	40	39	40	94	94	94	n
23	0	0	0	0	0	4	0	0	0	0	600	5	5	7	Min
1091	18	6909	770	0	800	235	1000	1	198	1000	1500	60	130	106	Max
520	0	720	315	0	0	12	300	0	0	516	800	11	82	15	Median
446	0	1028	327	0	0	11	291	0	0	518	817	12	81	16	Mean
31	-	93	38	-	-	15	42	-	-	8	8	21	8	14	CV (%)
0	1	0	0	0	4	0	0	1	9	0	0	0	0	0	False pos.
0	0	0	1	0	0	0	4	0	0	17	0	0	0	0	False neg.
0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	Outliers <
0	0	0	0	0	0	2	0	0	0	2	1	3	0	1	Outliers >
23	0	0	23	0	0	4	20	0	0	360	600	5	44	7	Low limit
1091	0	6909	770	0	0	16	1000	0	0	700	1132	33	130	31	High limit

21,129	0,000	32,063	18,091	0,000	0,000	3,335	17,061	0,000	0,000	22,750	28,576	3,487	9,025	3,938	mv
6,534	0,000	29,822	6,843	0,000	0,000	0,512	7,166	0,000	0,000	1,799	2,329	0,735	0,757	0,540	s
															x
															z

**Annex B** Z-values calculated from the laboratory results. Susp. = Suspected on the membrane filters before confirmation.  $z = (x - \bar{m}) / s$ . Z-values are calculated also for outliers (including false negative results) in the same way as ordinary z-values. From false positive results can no z-values be calculated. Z-values from outliers are not real z-values

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
1131	3 2 1				-0.934	-1.328	1.309				-0.964	-1.259	0.000	-0.164	1.430	0.113	0.064	1.534	0.000
1132	3 2 1							-1.679	-1.473*	0.000	-0.712	1.104	-0.475	-0.881	1.200	0.000			
1149	3 1 2																		
1237	1 3 2				-0.358		0.575				0.366		0.000	<b>-2.479</b>		-0.214			
1254	3 2 1				-0.254	-0.596	-1.118				0.119	-0.529	0.000				-0.479	-0.150	0.000
1545	3 1 2				-0.358		0.948				0.366		0.000						
1594	2 1 3				<b>2.633</b>	-0.934	-0.215				1.915	-0.697	0.000	0.921	0.993	0.808	0.638	1.086	0.000
1611	1 2 3				1.466	-0.934	-0.215				0.366	-0.697	0.000	0.367	0.043	0.011	0.460	0.113	0.000
1753	3 2 1				-0.275	-0.111	0.815				0.558	0.479	0.000	-1.120	1.641	-0.159	-0.252	1.534	0.000
1868	1 3 2				-0.379	-0.202	-0.052				0.317	0.349	0.000	0.741	0.291	0.903	1.154	0.368	0.000
1970	2 3 1				-0.055	<b>3.030</b>	0.187				0.119	-0.368	0.000						
2050	2 3 1				-0.337	-0.202	0.710				0.414	0.349	0.000	0.748	0.043	<b>2.139</b>	0.249	0.113	0.000
2386	3 1 2				-0.690	<b>2.043</b>	1.309				-0.848	<b>3.558</b>	0.000						
2637	3 1 2													-1.161	0.532	1.018	-1.707	0.614	0.000
2704	3 1 2				-0.153	-0.393	-1.073				0.838	0.077		0.189	0.291	0.452	-1.195	0.368	0.000
2745	1 3 2				-0.153	-1.477	-1.535				0.838	-1.473							
3042	3 1 2				1.861	0.759	-0.634				0.366	0.077		<b>4.000</b>	-0.756	0.448	-1.707	-1.001	0.000
3055	3 2 1													<b>2.265</b>	<b>4.000</b>	<b>-2.640</b>			
3076	2 3 1																		
3159	2 1 3													-1.080	0.719	-0.085	-0.316	0.467	0.000
3162	2 1 3				-0.306	0.064	0.536				0.487	0.729	0.000	-0.164	0.291	-0.830	1.349	0.368	0.000
3305	3 1 2													<b>-4.000</b>	-1.346	<b>-4.000</b>	<b>-4.000</b>	-1.635	0.000
3339	3 1 2				0.133	-0.493	-1.073				1.500	-0.066	0.000	0.238	0.993	-0.078	1.070	1.086	0.000
3475	2 1 3				-0.411	-0.493	-1.577				0.243	-0.066	0.000	0.778	-1.762	1.198	-1.411	-1.736	0.000
3511	3 2 1													-1.203	-0.697	0.000			
3588	2 3 1				-0.254	-0.493	0.609												
3730	2 1 3				-0.254	0.064	1.309				0.605	0.729	0.000	0.359	0.766	0.485	-0.644	0.854	0.000
3868	1 3 2				-0.822	-0.817	-0.617				-0.705	-1.060	0.000	-1.980	-1.346	-0.082	-0.881	-1.310	0.000
4015	3 1 2				<b>1.823</b>	<b>2.892</b>	0.286				1.647	<b>4.000</b>							
4064	2 3 1				-0.809	-0.296	0.383				-0.677	0.215	0.000						
4180	2 3 1				<b>-2.696</b>	1.824													
4278	2 1 3				-0.998	<b>4.000</b>	-0.634												
4288	3 2 1				-0.644	-0.393	0.180				0.884	0.349	0.000	-1.110	0.291	-0.797	-0.413	0.368	0.000
4319	3 2 1				-0.134	-0.202	1.277				0.888	<b>3.082</b>	0.000	-1.060	0.766	1.360	0.310	0.854	0.000
4343	1 3 2				-0.153	1.710	0.985				-1.914	-0.697		0.898	0.766	-0.078	1.782	0.368	0.000
4356	2 3 1				-1.345	<b>4.000</b>	<b>-2.068</b>							-0.903	0.291	-0.918	0.549	0.113	0.000
4459	1 2 3													-0.321	-0.368	0.000	-0.545	-0.706	0.000
4539	3 1 2				-0.422	0.148	0.223							<b>-4.000</b>	<b>-2.068</b>	<b>-4.000</b>	<b>-4.000</b>	<b>-2.050</b>	0.000
4633	1 3 2													-0.677	-0.214	0.000	-0.769	-1.999	0.301
4689	2 3 1				1.667	-0.596	-0.215				0.676	1.621	0.000				-0.644	-1.979	0.000
4713	3 1 2				-0.224	0.688	-0.215				-1.914	0.215	0.000						
4723	1 2 3				-0.809	<b>4.000</b>	-0.764							-0.137	0.000	<b>0.898</b>	<b>-2.739</b>	-0.078	0.002
4859	3 2 1				-0.576		1.309				-0.994	-0.697	0.000	0.189	-0.292	-0.918	-0.495	-0.231	0.000
4889	3 1 2				-0.797	0.148	<b>-2.489</b>				-0.677	1.412	0.000	1.562	0.993	-0.459	0.218	1.086	0.000
4980	1 2 3				-0.809	0.541	-0.215							-1.023	-1.060	0.000			
5018	2 1 3				<b>2.230</b>	<b>2.150</b>	-0.634							-0.119	-0.697	0.000			
5094	2 3 1				1.601	-0.934	-0.215							<b>2.036</b>	0.479	0.000			
5220	1 3 2													-0.566	-0.368	0.000	0.359	-0.480	-1.218
5352	1 2 3				-0.871	-0.493	-0.012							-0.964	-0.368	0.000	0.755	-0.422	0.000
5447	1 3 2				0.365	-0.111	-0.289												
5553	3 2 1				-0.621	0.541	-1.073							-0.572	-0.214	1.444	0.460	-0.150	0.000
5950	1 2 3				-0.576	-0.023	0.575				0.605	0.606	0.000	1.805	0.043	1.656	-1.375	0.113	0.000
6180	3 1 2				<b>2.577</b>	-0.393	-1.535				1.285	0.077	0.000	0.980	0.043	-0.839	0.549	0.113	0.000
6253	1 3 2													-0.913	1.641	1.293	-0.316	1.750	0.000
6456	1 2 3				-0.871	-0.493	-0.012							-0.964	-0.368	0.000	0.755	-0.422	0.000
6731	1 3 2													-0.293	-0.066	0.000	0.293	-0.214	0.000
7096	2 1 3				-0.390	-0.493	-0.133				-1.265	<b>4.000</b>	0.000	<b>2.057</b>	-0.025	0.787	<b>2.625</b>	0.043	0.000
7248	1 2 3				-0.610	<b>2.306</b>	0.258				1.854	0.729	0.000	0.446	-0.214	0.128	0.400	-0.150	0.000
7302	3 2 1				0.286	0.064	-0.751				0.119	0.479	0.000	-0.164	0.291	-0.547	0.667	0.368	0.000
7442	1 2 3				-0.465	-0.111	-0.215												
7533	2 1 3																		
7596	3 2 1				0.224	-0.817	-0.464				-0.877	-1.060	0.000	-1.201	1.641	-0.471	-0.950	1.750	0.000
7626	1 3 2				0.485	-0.023	-0.012				-0.163	0.606	0.000	0.710	1.430	1.008	0.002	1.534	0.000
7688	1 3 2				1.987	0.064	1.999				-0.677	0.729	0.000	0.657	0.532	0.352	0.579	0.614	0.000
7728	1 3 2				-0.173	-0.023	1.486				0.792	0.606		0.119	-1.060	0.000	-0.137	-0.697	0.000
7876	3 1 2				-0.465	-0.023	0.108							-0.321	1.214	-0.982	0.310	1.313	0.000
7896	1 2 3													-0.964	-0.874	0.000	0.649	-0.214	0.378
7930	2 1 3				<b>2.170</b>	-1.058	0.305										<b>4.000</b>	-0.150	0.000
7946	2 1 3																		
7962	3 2 1				-0.358	<b>3.385</b>	<b>2.330</b>				0.366	<b>4.000</b>		0.238	-0.214	-1.110	1.070	-0.150	0.000
7968	2 3 1																		

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)			C. perfringens (MF)			Mould (MF)			Yeast (MF)			Total plate count 22 °C, 3 days			Lab no.			
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C				
1.332	0.000	-1.075	-1.345	0.000	0.000	*			0.793	-1.087	0.000	0.000	1.417	0.467	1.340	-0.405	0.564	1131
									0.793	-1.189	0.000	0.000	-0.217	0.175	-0.031	0.041	-0.120	1132
0.873	0.000	0.762	1.277	0.000	0.000	0.793	-1.087	0.000	0.000	1.417	0.467	0.525	0.186	-0.615	1254			
0.503	0.000	-0.922				0.793	-1.189	0.000	0.000	-0.217	0.175	0.525	0.186	-0.615	1237			
0.084	0.000	1.098	0.524	0.000	0.000	1.298	0.892	0.000	0.000	-0.472	-0.472	-0.662	0.329	-0.120	1545			
-0.290	0.000	0.644				0.252	-0.672	0.000	-0.044	2.177	-0.662	-0.330	0.564	1594				
			-0.577	0.000	0.000	0.528	-1.544	0.000	-0.442	1.369	-0.662	-1.948	-0.120	1611				
			-1.512	0.000	0.000	0.011	0.000	0.000	-0.442	1.369	0.525	0.749	-0.120	1753				
											-0.896	-2.487	0.779	1868				
											-0.662	-0.405	-1.736	2050				
											-0.896	0.680	-0.615	2386				
											2.058	0.541	-1.150	2637				
											0.525	3.140	-0.615	2704				
-1.509	0.000	-1.075	-0.767	0.000	0.000	0.793	0.612	0.000	0.000	0.100	1.340	-0.481	0.115	2745				
											4.000	-0.106	-1.436	3055				
											-1.144	-0.405	-1.436	3076				
											-0.232	1.420	0.779	3159				
-0.496	0.000	-0.227				0.528	-0.985	0.000	0.000	-0.468	-1.579	-0.441	-0.712	-0.363	3162			
-1.238	0.000	-1.075				0.591	0.000	0.000				-1.701	0.886	0.779	3305			
0.154		-0.815				0.000						1.028	0.186	-0.615	3339			
												1.491	0.611	-0.120	3475			
												1.637	-0.033	-0.877	3511			
												-0.662	0.680	-0.877	3588			
												-0.031	1.354	0.115	3730			
0.256	0.000	-1.075	0.689	0.000	0.000	-0.036	0.000	0.000	4.000	-0.050	0.698	-0.106	-0.120	3868				
1.191	0.000	1.111				-0.654	-1.125	0.000	0.000	0.005	-1.527	-0.662	0.329	0.564	4015			
												0.347	-1.109	0.115	4064			
												0.162	-0.330	-0.877	4180			
												-0.662	0.954	-0.120	4278			
												-0.232	0.611	3.017	4288			
												-0.662	-0.405	0.989	4319			
0.772	0.000	-0.295	-0.427	0.000	0.000	0.528	0.933	0.000			-0.332	0.866	0.186	0.564	4343			
-0.766	0.000	1.296				-0.755	0.000	0.000				-1.701	0.818	0.989	4356			
0.339	0.000	0.699										-0.232	-0.106	-0.615	4459			
1.030	0.000	-1.075										0.347	-0.635	2.506	4539			
0.401	0.000	1.422										-1.144	0.680	-1.736	4633			
												-0.232	-1.861	1.966	4689			
												0.347	-0.180	-0.363	4713			
												0.866	1.022	-0.120	4723			
												-0.896	-0.558	-0.363	4889			
												-0.232	-1.190	-0.615	4980			
												0.290	0.000	0.336	5018			
												-1.753	-0.232	-1.109	0.989	5094		
												-1.411	-4.000	0.564	5220			
												-0.896	-0.481	-0.120	5352			
0.867	0.000	1.690	1.272	0.000	0.000	0.528	2.032	0.000	0.000	0.741	0.100	-0.232	1.354	-0.120	5447			
			0.936	0.000	0.000					-0.693		1.491	-0.106	1.588	5553			
-0.250	0.000	1.199	0.205	0.000	0.000	-2.605	-0.407	0.000	0.030	-0.433		-0.896	0.041	0.343	5950			
0.930	0.000	1.072	1.332	0.000	0.000							-0.232	-0.481	-0.120	6180			
												0.662	-0.481	-0.120	6253			
												-1.701	0.541	-0.120	6456			
												0.162	0.541	-0.120	6731			
												-1.144	1.022	-2.392	7096			
0.958	0.000	0.324	1.195	0.000		-0.654	0.986	0.000			0.647	-0.441	-0.948	0.115	7248			
-0.790	0.000	-0.889	-0.310	0.000		-0.337	0.036	0.000	0.000	0.970	-1.753	-0.662	-1.109	0.343	7302			
0.626	0.000	0.822										-0.441	-0.255	1.779	7442			
															7533			
															7596			
-1.688	0.000	-1.075	-1.168	0.000	0.000	4.000	0.811	0.000	0.000	-0.194		-0.031	-0.791	-0.120	7626			
0.647	0.000	-0.020	1.062	0.000	0.000	1.195	-0.730	0.000	0.000	2.061	0.964	-1.144	1.996	-0.120	7688			
												0.698	-0.558	-0.120	7728			
												0.420	0.000	-0.757	7876			
												1.050	1.591	0.000	7896			
												0.698	0.471	1.966	7930			
												4.000	-3.159	4.000	7946			
												-0.036	1.231	0.000	7962			
												1.298	-1.757	0.000	7968			
												0.000	-0.660	-0.371	8068			
												-0.654	-0.407	0.000	8177			
												0.000	-2.100	-0.590	8255			
												0.611	0.637	-0.791	8260			
												0.000	0.801	0.000	8329			
												0.528	1.298	0.000	8380			
												0.000	-0.789	0.113	8435			
												0.611	0.637	-0.791	8455			
												0.000	-0.232	-1.604	8463			
												0.000	0.126	0.000	8569			
												0.000	0.028	0.343	8598			
												0.000	1.340	-0.869	0.564	8626		
												0.000	3.071	-1.028	0.779	8628		
												-0.232	0.400	-0.877	8663			

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
8742	1 3 2																		
8751	2 3 1																		
8766	2 3 1				-0.520	-0.296	-0.215				-0.008	0.215	0.000	0.238	-1.044	0.352	0.064	-1.001	0.000
8862	1 3 2				-0.153	-0.817	0.152				0.838	-0.529	0.000	-0.536	0.291	0.854	-0.156	0.368	0.000
8898	1 2 3				<b>2.200</b>	-0.596	0.456				1.478	-0.214	0.000	0.579	-0.214	1.451	1.070	-0.150	0.000
8955	3 1 2				0.040	1.475	-0.634				1.285	<b>2.746</b>	0.000	0.672	-0.480	1.018	1.728	-0.422	0.000
9002	3 2 1				0.040	1.475	-1.073				-1.914	-1.060	0.000						
9359	3 1 2				-0.809	-0.111	-0.215				0.119	0.479	0.000	-0.138	0.043	-0.078	-1.339	0.113	0.000
9436	3 2 1				-0.834	-1.058	-1.237				-0.734	-0.874	0.000	-1.357	0.291	-1.398	-0.712	0.368	0.000
9451	2 1 3				-0.576	0.829	0.575				-0.877	-1.259	0.000						
9465	3 2 1				1.271	-0.393	-0.105				-0.008	0.077	0.000	0.876	0.291	0.352	0.957	0.368	0.000
9569	2 1 3				1.257	-1.058	1.762				-0.402	-0.874	0.000	-0.356	0.043	0.352	-0.712	-0.150	0.000
9589	2 3 1				0.400	-1.328	-0.634				0.884	-0.368	0.000	<b>-2.027</b>	0.043	-0.459	<b>-2.884</b>	0.113	0.000
9736	1 3 2				-0.134	-0.704	-0.335				1.854	-0.529	0.000						
9899	1 2 3				0.286	-0.817	0.896												
9903	3 1 2				0.021	-0.111	-0.149				-0.137	-0.066	0.000						

n		0	0	0	79	75	78	0	0	0	77	73	70	60	61	59	59	59	60
Min					-2.696	-1.477	-2.542				-2.344	-1.473	0.000	-4.000	-2.739	-4.000	-4.000	-2.738	0.000
Max					2.633	4.000	2.330				2.036	4.000	0.000	4.000	4.000	2.139	4.000	1.750	0.000
Median					-0.254	-0.111	-0.032				0.119	-0.066	0.000	0.189	0.043	-0.078	0.002	0.113	0.000
Mean					0.000	0.205	0.000				0.000	0.213	0.000	-0.067	0.066	-0.136	-0.136	0.000	0.000
SD					1.000	1.306	1.000				1.000	1.319	0.000	1.326	1.116	1.224	1.420	1.000	0.000
<b>z&lt;-3</b>					0	0	0				0	0	0	2	0	2	3	0	0
<b>-3&lt;z&lt;-2</b>					1	0	3				1	0	0	2	2	3	1	2	0
<b>2&lt;z&lt;3</b>					5	4	1				1	3	0	2	0	1	1	0	0
<b>z&gt;3</b>					0	5	0				0	5	0	1	1	0	1	0	0

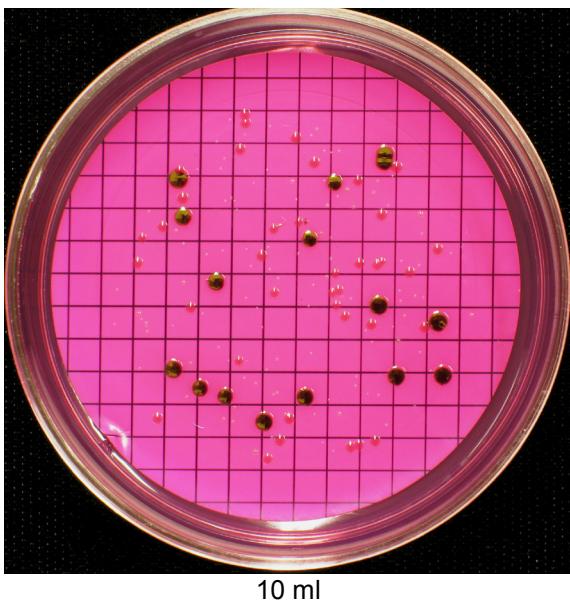
Presumptive <i>C. perfringens</i> (MF)			<i>C. perfringens</i> (MF)			Mould (MF)			Yeast (MF)			Total plate count 22 °C, 3 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
-0.705	0.000	1.296				0.793	0.503	0.000	0.000	-0.110	-1.144	-0.255	-1.736	8742	
1.021	0.000	-1.075				1.298	-0.917	0.000	0.000	-1.655	-0.837	0.162	<b>-4.000</b>	<b>-2.054</b>	8751
0.722	0.000	-0.521				-0.036	-0.778	0.000	0.000	0.331	-0.749	-0.031	0.541	1.193	8766
			0.473	0.000	0.000	<b>-2.145</b>	-0.509		0.000	0.625	-0.126	-0.662	0.258	-1.436	8862
												-1.411	-0.180	0.564	8898
1.013	0.000	0.667	1.411	0.000	0.000	-0.654	-0.985	0.000	0.000	-0.724	-0.050	<b>3.419</b>	0.749	-0.615	9002
-0.790	0.000	1.501				-0.989	1.012	0.000	0.000		-0.837	1.491	0.400	-0.877	9359
-0.583	0.000	-0.348	-0.113	0.000	0.000							0.866	0.471	1.393	9436
												0.162	0.113	<b>-2.054</b>	9451
<b>-2.500</b>	0.000	-0.127	-1.943	0.000		0.793	1.066	0.000	0.000	-0.991	1.921	1.288	0.115	9465	9569
-0.124	0.000	1.140				1.050	0.036	0.000			<b>4.000</b>	-1.144	0.113	-0.120	9589
-0.086	0.000	1.712				-0.654	-0.992	0.000	0.000	0.602	1.490	0.525	-0.405	1.393	9736
0.398	0.000	-0.993				0.528	0.516	0.000	0.000		1.349	-0.662	0.041	-0.120	9899
															9903
43	43	44	34	36	32	39	36	38	31	22	40	94	94	94	n
-2.500	0.000	-1.075	-1.943	0.000	0.000	-2.605	-1.757	0.000	0.000	-2.100	-1.753	-1.701	-4.000	-2.392	Min
1.821	0.000	1.712	1.411	0.000	0.000	4.000	2.032	0.000	0.000	4.000	4.000	4.000	3.140	4.000	Max
0.256	0.000	-0.177	-0.051	0.000	0.000	0.528	0.036	0.000	0.000	0.017	-0.118	-0.232	0.041	-0.120	Median
0.000	0.000	0.000	0.000	0.000	0.000	0.205	0.000	0.000	0.000	0.364	0.100	0.121	-0.085	0.043	Mean
1.000	0.000	1.000	1.000	0.000	0.000	1.321	1.000	0.000	0.000	1.513	1.172	1.193	1.147	1.077	SD
0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	Summa
2	0	0	0	0	0	3	0	0	0	1	0	0	2	3	10
0	0	0	0	0	0	0	1	0	0	1	1	2	0	1	26
0	0	0	0	0	0	2	0	0	0	2	1	4	2	2	24
															26

## Annex C – photos

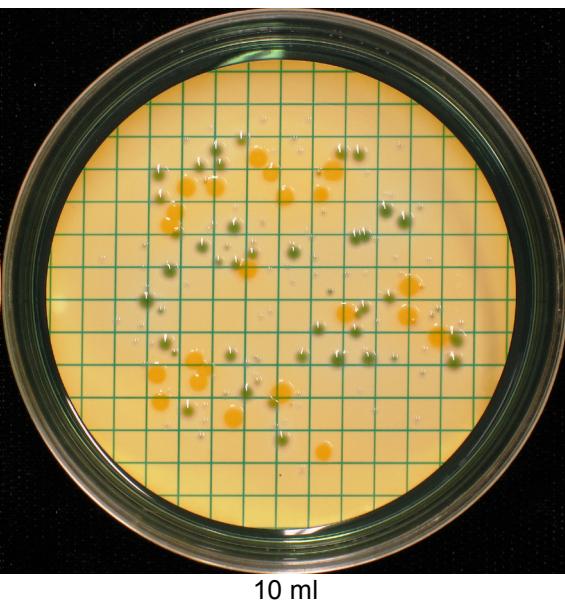
Drinking water, March 2012

Mixture A

m-Endo Agar LFS, 37 °C



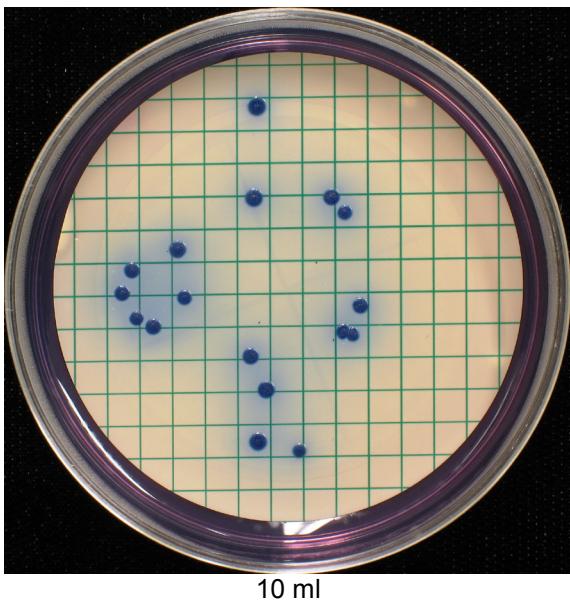
10 ml



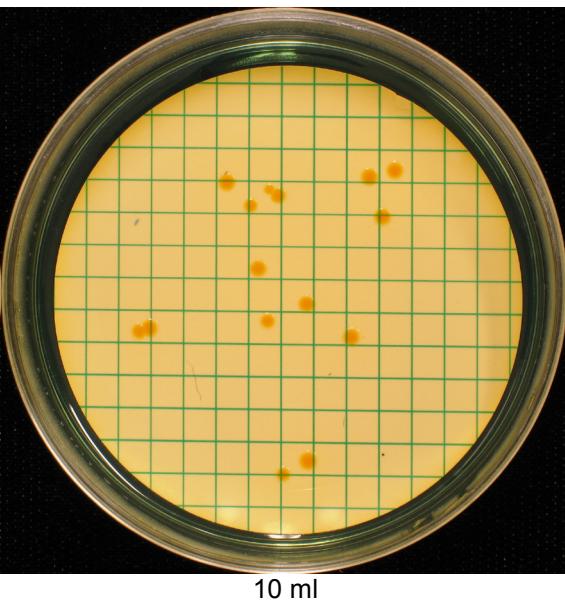
10 ml

m-Lactose TTC Agar, 37 °C

m-FCC Agar, 44 °C



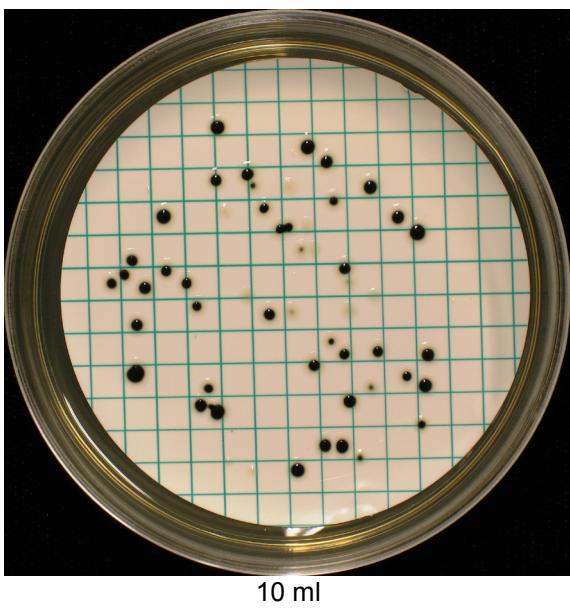
10 ml



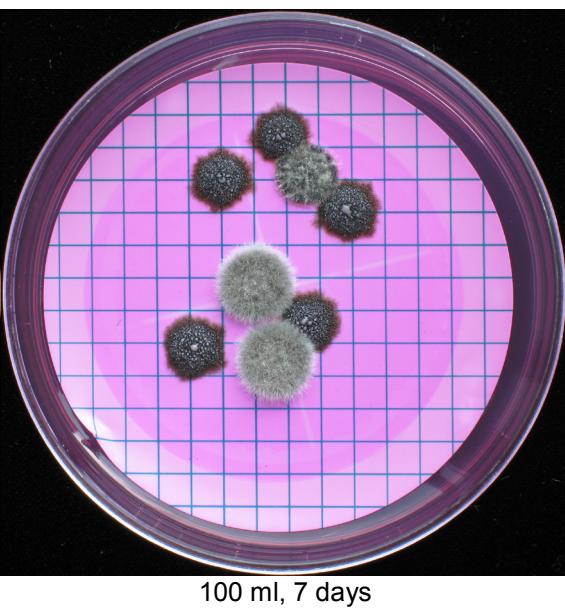
10 ml

m-Lactose TTC Agar, 44 °C

m-TSC Agar, 44 °C



10 ml

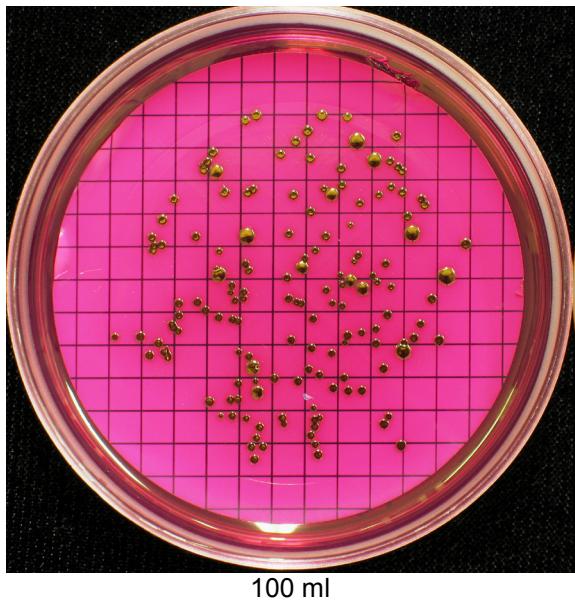


100 ml, 7 days

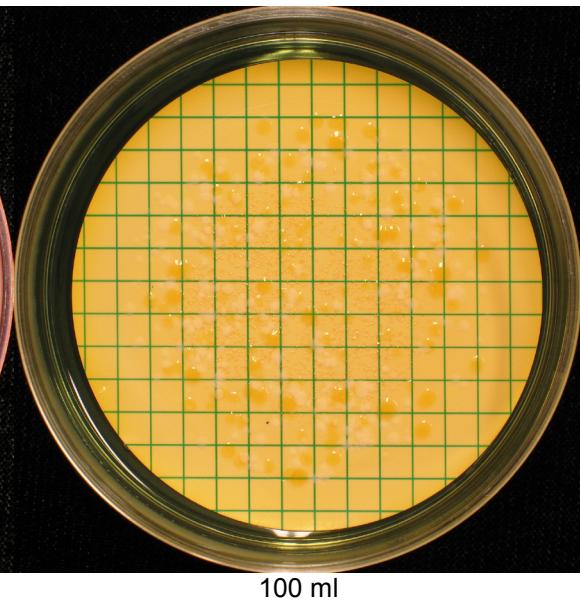
m-Burman Agar, 25 °C

## Mixture B

m-Endo Agar LEE, 37 °C

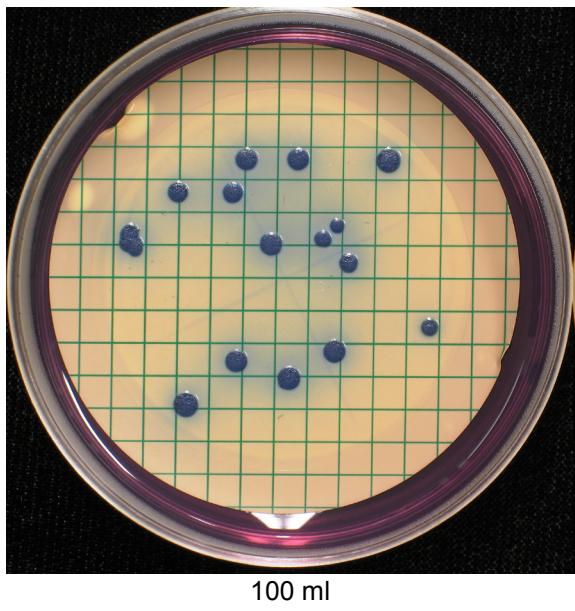


100 ml

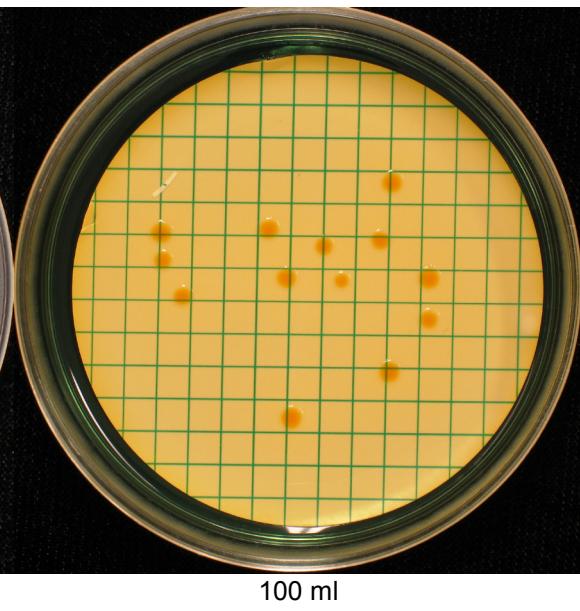


100 ml

m-FCC Agar, 44 °C

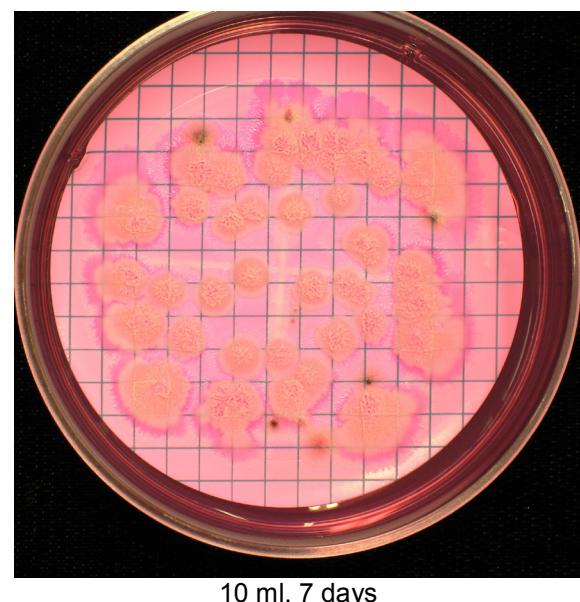


100 ml



100 ml

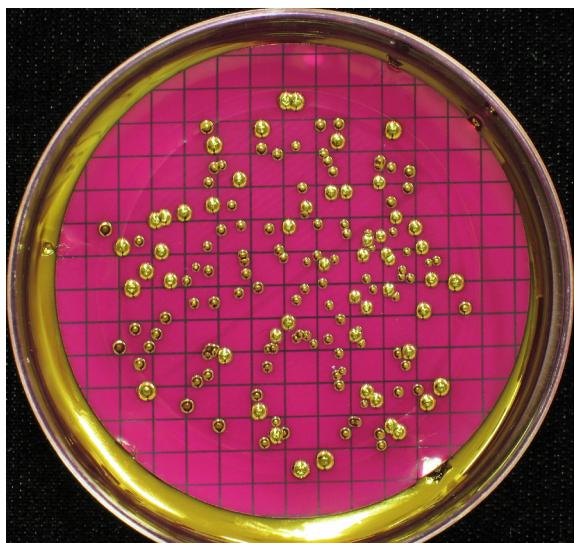
m-TSC Agar, 44 °C



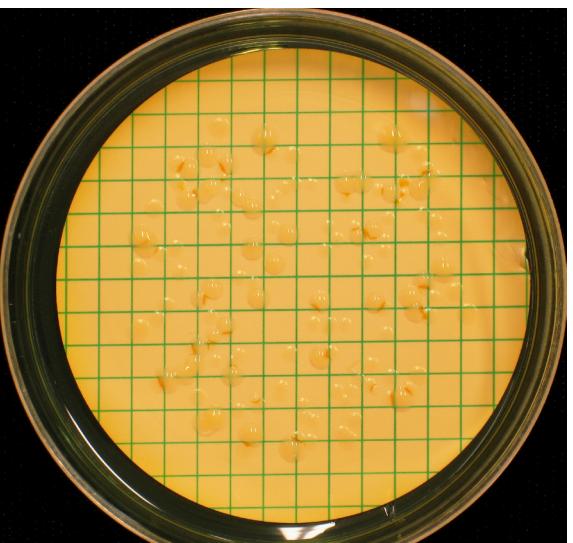
10 ml, 7 days

### Mixture C

m-Endo Agar L<sub>ES</sub>, 37 °C

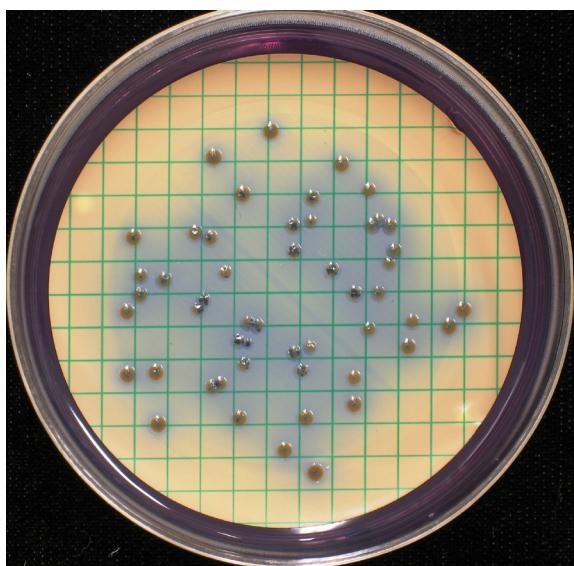


10 ml

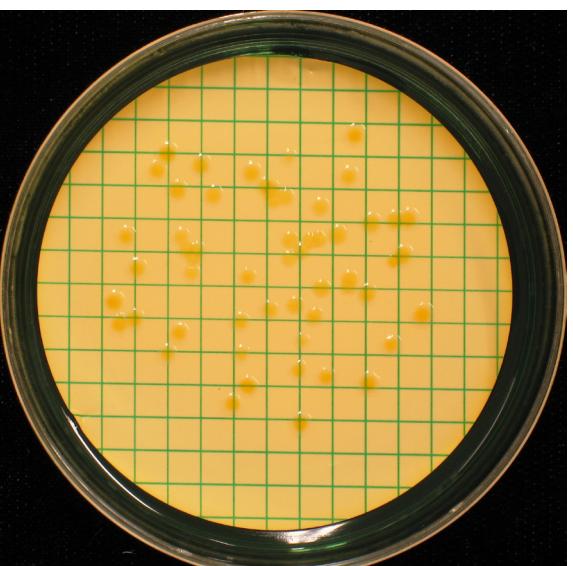


10 ml

m-TFC Agar, 44 °C

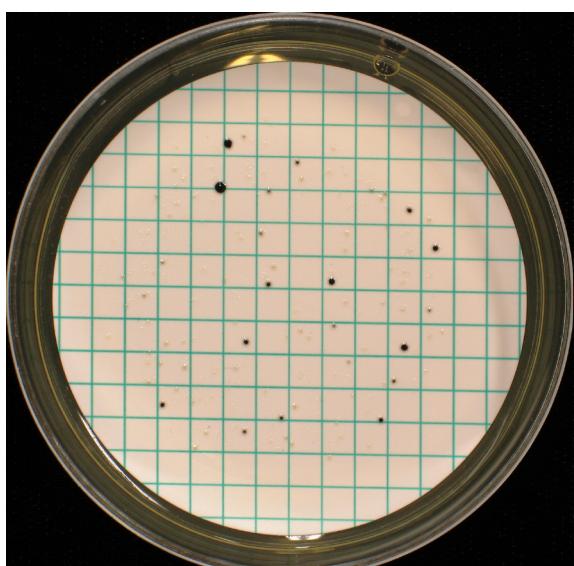


10 ml

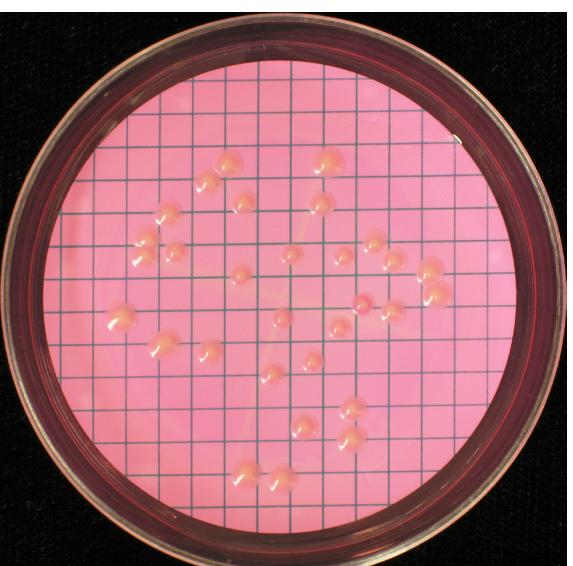


10 ml

m-TSC Agar, 44 °C



100 ml



5 ml, 7 days

m-Lactose TTC Agar, 37 °C

m-Lactose TTC Agar, 44 °C

m-Burman Agar, 25 °C

1. Lunch och lärande – skollunchens betydelse för elevernas prestation och situation i klassrummet av M Lennernäs.
2. Kosttillskott som säljs via Internet – en studie av hur kraven i lagstiftningen uppfylls av A Wedholm Pallas, A Laser Reuterswärd och U Beckman-Sundh.
3. Vetenskapligt underlag till råd om bra mat i äldreomsorgen. Sammanställt av E Lövestram.
4. Livsmedelssvinn i hushåll och skolor – en kunskapsammanställning av R Modin.
5. Riskprofil för material i kontakt med livsmedel av K Svensson, Livsmedelsverket och G Olafsson, Rikisendurskodun (Environmental and Food Agency of Iceland).
6. Proficiency Testing – Food Microbiology, January 2011 by C Normark and I Boriak
7. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N 47.
8. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T-22 by C Åstrand and L Jorhem.
9. Riksprojekt 2010. Listeria monocytogenes i kyld ätfärdig mat av C Nilsson och M Lindblad.
10. Kontroll av restsubstanser i levande djur och animaliska livsmedel. Resultat 2010 av I Nordlander, Å Kjellgren, A Glynn, , B Aspenström-Fagerlund, K Granelli, I Nilsson, C Sjölund Livsmedelsverket och K Girma, Jordbruksverket.
11. Proficiency Testing – Food Microbiology, April 2011 by C Normark, I Boriak, M Lindqvist and I Tillander.
12. Bär – analys av näringssämnena av V Öhrvik, I Mattisson, A Staffas och H S Strandler.
13. Proficiency Testing – Drinking Water Microbiology, 2011:1, March by T Slapokas, C Lantz and M Lindqvist.
14. Kontrollprogrammet för tvåskaliga blötdjur – Årsrapport 2009-2010 – av av I Nordlander, M Persson, H Hallström, M Simonsson, Livsmedelsverket och B Karlsson, SMHI.
15. Margariner och matfettsblandningar – analys av fettsyror av R Åsgård och S Wretling.
16. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N 48.
17. Kontroll av bekämpningsmedelsrester i livsmedel 2009 av A Jansson, X Holmbäck och A Wannberg.
18. Klimatpåverkan och energianvändning från livsmedelsförpackningar av M Wallman och K Nilsson.
19. Klimatpåverkan i kylkedjan – från livsmedelsindustri till konsument av K Nilsson och U Lindberg.
20. Förvara maten rätt så håller den längre – vetenskapligt underlag om optimal förvaring av livsmedel av R Modin och M Lindblad.
21. Råd om mat för barn 0-5 år. Vetenskapligt underlag med risk- och nyttovärderingar och kunskapsöversikter.
22. Råd om mat för barn 0-5 år. Hanteringsrapport som beskriver hur risk- och nyttovärderingar, tillsammans med andra faktorer, har lett fram till Livsmedelsverkets råd.
23. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T-23 by C Åstrand and Lars Jorhem.
24. Proficiency Testing – Food Chemistry, Vitamins in Food, Round V-9 by A Staffas and H S Strandler.
25. Nordiskt kontrollprojekt om nyckelhålmärkning 2011 av I Lindeberg.
26. Rapport från GMO-projektet 2011. Undersökning av förekomsten av GMO i livsmedel av Z Kurowska.
27. Fat Quality - Trends in fatty acid composition over the last decade by I Mattisson, S Trattner and S Wretling.
28. Proficiency Testing – Drinking Water Microbiology, 2011:2, September by T Slapokas and M Lindqvist.
29. Kontrollen roll skiljer sig mellan livsmedelsbranscherna av T Ahlström, G Jansson och S Sylvén.
30. Kommuner och Livsmedelsverkets rapportering av livsmedelskontrollen 2011 av C Svärd och L Eskilsson.
31. Proficiency Testing – Food Microbiology, October 2011 by C Normark and I Boriak.

1. Fisk, skaldjur och fiskprodukter – analys av näringssämen av V Öhrvik, A von Malmborg, I Mattisson, S Wretling och C Åstrand.
2. Normerande kontroll av dricksvattenanläggningar 2007-2010 av T Lindberg.
3. Tidstrender av tungmetaller och organiska klorerade miljöföroringar i baslivsmedel av J Ålander, I Nilsson, B Sundström, L Jorhem, I Nordlander, M Aune, L Larsson, J Kuivinen, A Bergh, M Isaksson och A Glynn.
4. Proficiency Testing – Food Microbiology, October 2011 by C Normark, I Boriak and L Nachin.
5. Mögel och mögelgifter i torkad frukt av E Fredlund och J Spång.
6. Mikrobiologiska dricksvattenrisker ur ett kretsloppsperspektiv – behov och åtgärder av R Dryselius.
7. Market Basket 2010 – chemical analysis, exposure estimation and health-related assessment of nutrients and toxic compounds in Swedish food baskets.
8. Proficiency Testing – Food Microbiology, April 2012 by L Nachin, C Normark, I Boriak and I Tillander.
9. Kontroll av restsubstanser i levande djur och animaliska livsmedel. Resultat 2010 av I Nordlander, Å Kjellgren, A Glynn, B Aspenström-Fagerlund, K Granelli, I Nilsson, C Sjölund Livsmedelsverket och K Girma, Jordbruksverket.
10. Råd om fullkorn 2009 - bakgrund och vetenskapligt underlag av W Becker, L Busk, I Mattisson och S Sand.
11. Nordiskt kontrollprojekt 2012. Märkning av allergener och ”kan innehålla spår av allergener” – resultat av de svenska kontrollerna.
12. Proficiency Testing – Drinking Water Microbiology, 2012:1, March by T Slapokas, M Lindqvist and K Mykkänen.