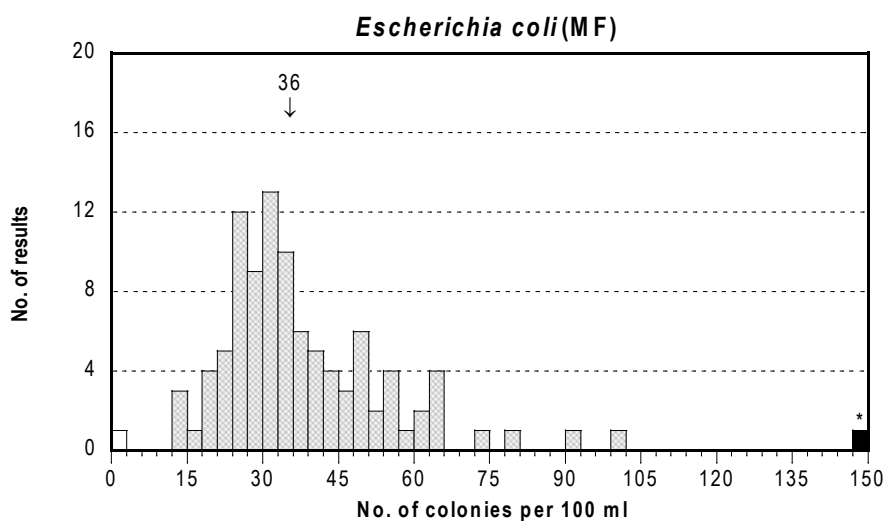


## Proficiency Testing

# Drinking Water Microbiology

## 2011:1, March

by Tommy Šlapokas, Christina Lantz and Malin Lindqvist





*Proficiency Testing*  
**Drinking Water Microbiology**  
– 2011:1, March –

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1<sup>st</sup> edition

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## Introduction

In all analytical activity it is of utmost importance that the work maintains a well documented high standard. Therefore, most laboratories have a system for quality assurance. How well this works has to be evaluated by an independent part. Such an external quality check of laboratory competence is also commonly required by accreditation bodies. One way is by taking part in proficiency tests (PT).

In a PT round test items are analysed by a number of laboratories. They are supposed to follow instructions, perform analyses using their routine methods and report their results to the organiser. The organiser evaluates the results and finally compiles them in a report.

### *Objectives of microbiological PT schemes at the National Food Administration*

1. Laboratories should receive an external evaluation of parts of their analytical competence, including usage of methods, documentation and orderliness.
2. The accreditation bodies in respective countries should have a tool at inspections regarding new accreditation and maintenance of accreditation.
3. The laboratories and the organiser should receive increased knowledge regarding how well methods work, with respect to various types of organisms, at laboratories that on a routinely basis perform corresponding analyses.

## Design

### **Analyses and mixtures**

This particular proficiency test was performed during week 11 in March 2011, and is registered as no. 774/2011 at the National Food Administration, Uppsala.

Samples were sent to 109 laboratories out of which 35 Swedish, 36 Finnish, 25 from the other Nordic countries and 13 from the rest of the world. Three of the laboratories did not report results.

### *Assessed parameters*

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

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

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

*Clostridium perfringens* with MF

**Micro fungi (yeast and mould)** with MF

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

### *Not assessed parameters (background for interpretations and discussions)*

**Suspected coliform bacteria** and **Suspected thermotolerant coliform bacteria** with MF, typical colonies (before confirmation) at 35/36/37 °C and 44/44,5 °C, respectively, on relevant media.

The proficiency test comprised three simulated water samples. Each laboratory was assigned to perform the analyses according to its ordinary methods routinely used on drinking water samples. The test material is first and foremost adjusted to those 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, in general, as well as other similar methods.

Three freeze-dried test materials were produced from 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 laboratory received one vial of each mixture. The simulated water samples, 800 ml each, were prepared by dissolving the content of the vials in sterile dilution or rinsing agent. The composition in respective mixture is clear from **table 1**.

**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-165	≈20
	<i>Escherichia coli</i>	SLV-295	30
	<i>Enterobacter cloacae</i>	SLV-451	32
	<i>Clostridium perfringens</i>	SLV-442	34
	<i>Hanseniaspora uvarum</i>	CFSQE77	830
	<i>Cladosporium cladosporoides</i>	SLV-488	≈120
B	<i>Citrobacter freundii</i>	SLV-091	220
	<i>Clostridium perfringens</i>	SLV-442	3
	<i>Candida glabrata</i>	SLV-052	33
	<i>Stenotrophomonas maltophilia</i>	SLV-041	200*
C	<i>Escherichia coli</i>	SLV-082	640
	<i>Klebsiella oxytoca</i>	SLV-089	660
	<i>Phialophora malorum</i>	SLV-545	8
	<i>Pseudomonas fluorescens</i>	SLV-535	34*

1 For linkage between the randomised sample number and respective mixture, please see annex A

2 Based on results from duplicate analyses, performed at the National Food Administration, of 10 vials per mixture (table 2), The results from m-Endo Agar LES have been used for *E. coli* SLV-082, SLV-165 & *E. coli* SLV-295, *E. cloacae*, *K. oxytoca* and *C. freundii*; those from TSC Agar for *C. perfringens*; those from RBCC Agar for *H. uvarum*, *C. cladosporoides*, *C. glabrata* and *Ph. malorum*; those from YeA for *S. maltophilia* and *P. fluorescens* — no. is stated as cfu ("colony forming units") per 100 ml, if other is not stated

\* cfu per ml



## Quality check of the samples

Homogenous samples and uniform volumes in all vials are prerequisites in order for comparison of all freeze dried samples from one mixture to be feasible. The volume has been checked in 15 vials from each mixture. The differences between vials in the mixtures were 3-5 mg. The highest accepted variation is 15 mg (3%). **Table 2** presents the results from duplicate analyses of 10 vials from each mixture as coefficients of variation (CV). The results relate to that unit by volume at which the colonies were counted. According to the criteria used, the CV's were acceptable for all mixtures in order to be regarded as homogenous. The highest accepted CV is normally 25%. The CV for moulds in mixture A and *C. perfringens* in mixture B was high (> 25%). The reason is low mean values ( $\leq 4$  cfu/unit by volume) that make high CV acceptable. The result of culturable microorganisms in mixture A is mainly obtained from mould and yeast colonies, which might explain the relatively high CV. To read 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>	7	2	5 <sup>a</sup>
<i>Escherichia coli</i> (MF) <sup>3</sup>	8	—	5 <sup>a</sup>
Presumptive <i>Clostridium perfringens</i> <sup>4</sup>	10	26 <sup>*</sup>	—
Mould (MF) <sup>5</sup>	41 <sup>a*</sup>	—	9
Yeast (MF) <sup>5</sup>	5 <sup>a</sup>	9	—
Culturable microorg., 3d 22 °C (pour-plate) <sup>6</sup>	22	6	8

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 7, 8 and 11 weeks ahead of the testing week, respectively.

2 m-Endo Agar LES according to SS 028167 [analyses were also made on m-Lactose TTC Agar with Tergitol according to SS-EN ISO 9308-1:2000, but those results are not accounted for here]

3 m-FC Agar, 44 °C, according to SS 028167 [analyses were also made at 44 °C on Lactose TTC Agar with Tergitol acc. to SS-EN ISO 9308-1:2000, but those results are not accounted for here]

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 Read for the volume 10 ml

— No reading

\* High coefficient of variation due to very low mean value ( $\leq 4$  cfu/unit by volume)

## Laboratory results

### General information regarding the results

The histograms (**figure 1**) show the actual distribution of the results. False positives are not presented in the histograms. The total number of these, and other results with annotations, are compiled in **table 3**. False results and outliers are generally not included in the calculations. All reported laboratory results are compiled in **annex A** and photo examples of colony appearance are given in **annex B**. Z-values for the all evaluated results are given in **annex C**.

In most histograms are “tails” in either or both directions, representing values that do not belong to the distribution present. Better normal distributions are obtained by performing square root transformations of the results, and are, therefore, used for the calculations. The significance of these “tails” is in that way decreased. Very deviating values are identified as outliers also after square root transformation (black bars). They are present in most analyses. False negative results are presented as white bars.

Outliers are identified 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 itself is objective, it is a prerequisite that the results are normally distributed in order to obtain correct outliers at the 1% level. A result of zero that is identified as a low outlier is regarded as a false negative result. In special situations, e.g. when many zero results are reported and in some borderline cases, a few subjective adjustments are made in order to set just limits, based on the knowledge of the content of the mixtures. 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 <sup>1</sup>			Total	Total no. of laboratories
	A	B	C		
<i>No. of evaluated results</i>	57	579	579	<b>1735</b>	<b>106<sup>a</sup></b>
False positives	0	8	7	<b>15</b>	<b>11</b>
False negatives	12	2	3	<b>17</b>	<b>14</b>
Low outliers	9	3	13	<b>25</b>	<b>11</b>
High outliers	10	7	7	<b>24</b>	<b>16</b>
<i>No. of results with annotation</i>	31	20	30	<b>81</b>	<b>32<sup>b</sup></b>

1 Results obtained in the analyses designated suspected have not been included

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 mixture contained four bacterial strains and two fungi (table 1 and table 4): the coliform bacteria *E. coli* (2 strains) and *E. cloacae*, *C. perfringens*, the mould *C.*

**Table 4.** The outcome of each analysis in mixture A; F+ and F- represent the share (%) of false positive respectively false negative results. Outl < and Outl > represent the shares (%) of low and high outliers respectively. Shaded analyses are in general not numerically assessed - median is there 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>E. coli</i> MUG- <i>E. cloacae</i>	74	—				
Coliform bacteria (MF)	<i>E. coli</i> <i>E. coli</i> MUG- <i>E. aerogenes</i>	68	18	-	0	1	2
Susp. thermotol. colif. bact. (MF)	<i>E. coli</i> <i>E. coli</i> MUG- [ <i>E. cloacae</i> ]	40	—				
<i>E. coli</i> (MF)	<i>E. coli</i> { <i>E. coli</i> MUG-}	41	24	-	1	1	1
Coliform bact. (rapid method)	<i>E. coli</i> <i>E. coli</i> MUG- <i>E. cloacae</i>	78	13	-	0	3	3
<i>E. coli</i> (rapid method)	<i>E. coli</i>	19	18	-	0	0	0
Presumptive <i>C. perfringens</i> (MF)	<i>C. perfringens</i>	42	17	-	2	0	2
<i>C. perfringens</i> (MF)	<i>C. perfringens</i>	38	21	-	6	0	0
Mould (MF)	<i>C.</i> <i>cladosporoides</i>	116	28	-	7	0	2
Yeast (MF)	<i>H. uvarum</i>	854	9	-	0	2	0
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>H. uvarum</i> ( <i>E. cloacae</i> ) ( <i>E. coli</i> )	12	15	-	3	2	2

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

- numerical value impossible to obtain

— organism absent or numerical value has not been calculated

~ unreliable value since the results vary with different interpretations, method differences or such

() the organism contributes with very few colonies

[ ] the organism is false positive in a presumptive analysis

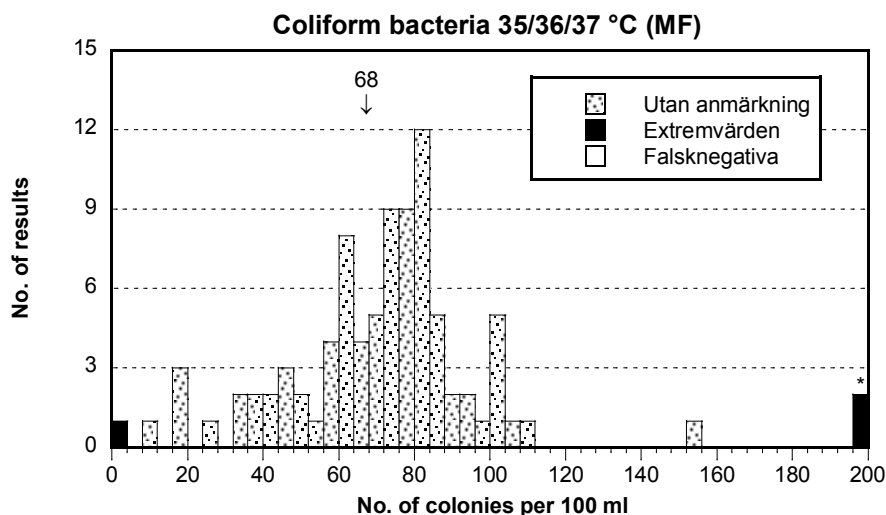
{ } the result depends on the definition

*cladosporoides* and the yeast *H. uvarum*. The yeast together with some individual colonies of the coliform bacteria and the mould may appear in the analysis of culturable microorganisms after 3 days at 22±2 °C.

The numbers of false positive and false negative results as well as low and high outliers are reported in annex A and their relative shares in table 4.

#### Coliform bacteria, MF

- The results were well distributed (figure 1A). The dispersion was small. However, a somewhat higher number of low results than expected occur.
- 1 low and 2 high outliers were reported.
- Two strains of *E. coli* and one of *E. cloacae* were present. The colonies were typical and easy to detect on m-Endo Agar LES. The colonies were relatively easy to distinguish even on m-Lactose TTC (LTTC) Agar, although the whole agar plate turned yellow.



**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 range of the x-axis has not been adjusted to very deviating high values. These values are marked with an asterisk (\*) and presented as outliers at the right end of x. 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.

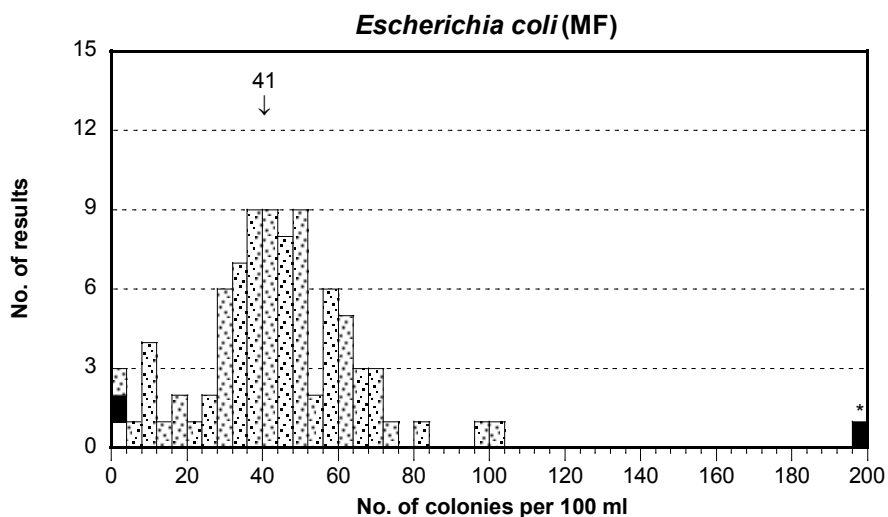
#### Suspected thermotolerant coliform bacteria, MF

Colonies regarded as suspected thermotolerant coliform bacteria were reported in 48 cases. The colonies were made up by the two strains of *E. coli*, which grows on m-FC Agar and LTTC Agar at 44/44.5 °C. Occasionally, small colonies of *E. cloacae* may also appear at 44 °C. For 18 laboratories the same number is reported

also for *E. coli* (MF), indicating that those results are reported from plates incubated at 44/44.5 °C.

#### *E. coli*, MF

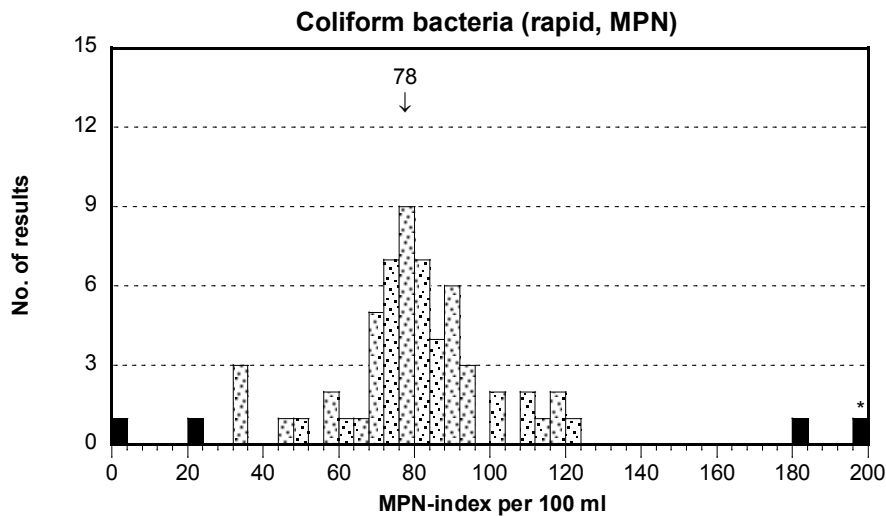
- The results were relatively well distributed (figure 1B) but with a somewhat higher number of low results than expected. The dispersion was medium.
- 1 false negative result and 1 low and 1 high outlier were reported.
- Colonies incubated on m-FC Agar or LTTC Agar at 44/44.5 °C were easy to detect and quite uniform. They represented the two strains of *E. coli*.
- When *E. coli* is determined by confirmation from plates incubated at 35-37 °C the results are a bit more variable than at 44/44.5 °C, because also colonies of *E. cloacae* grow at the lower temperature.
- When a chromogenic medium based on detection of  $\beta$ -glucuronidase activity is used (e.g. Chromocult Coliform Agar<sup>®</sup>) the MUG negative strain of *E. coli* will not be detected. This should imply lower *E. coli* result.



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

#### Coliform bacteria, rapid method

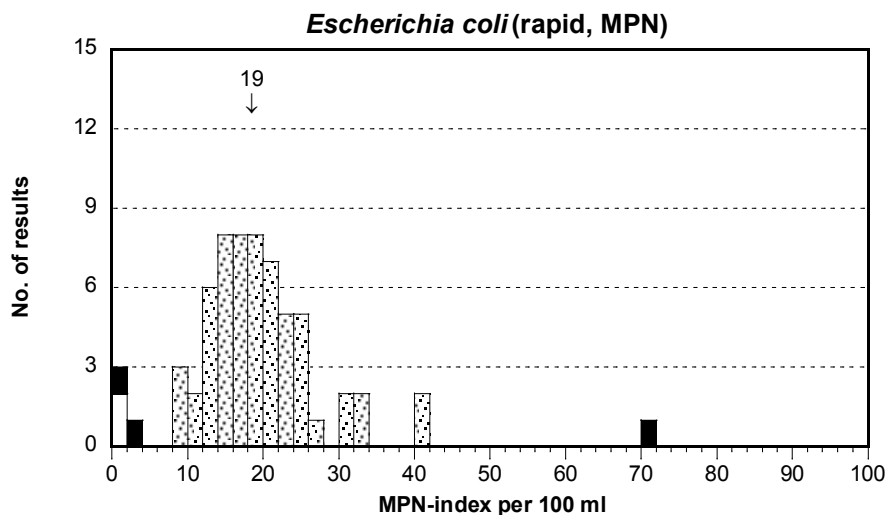
- The results were well distributed (figure 1C). The dispersion was small.
- 2 low and 2 high outliers were reported. No “tail” with low values was present, causing a somewhat higher mean content than with the MF method.
- All three coliform bacteria were detected.
- Almost only results obtained with Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup> were reported.



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

*E. coli*, rapid method

- The results were well distributed (figure 1D). The dispersion was small.
- The mean was lower than with the MF method. The reason is that the MUG negative strain of *E. coli* was not detected by methods based on activity of the enzyme  $\beta$ -glucuronidase, such as Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup> that was clearly the most used method.

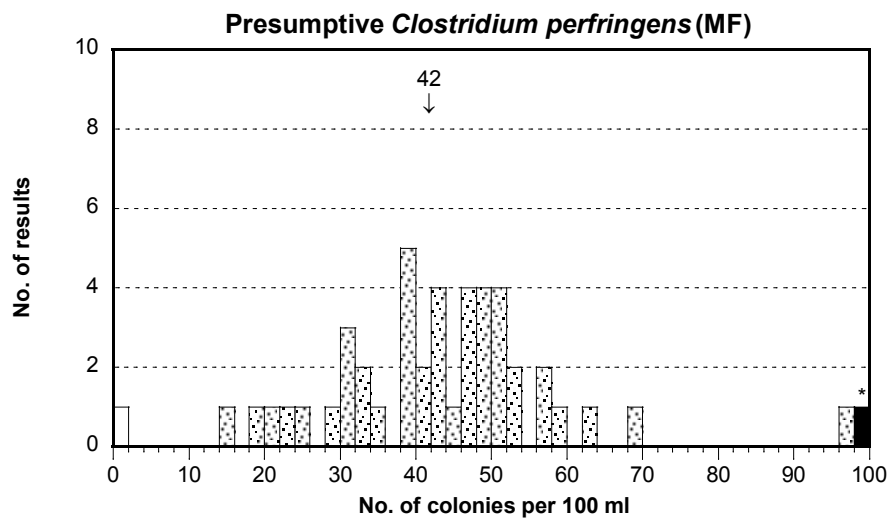


**Figure 1D** *Mixture A*, see figure 1A for explanations

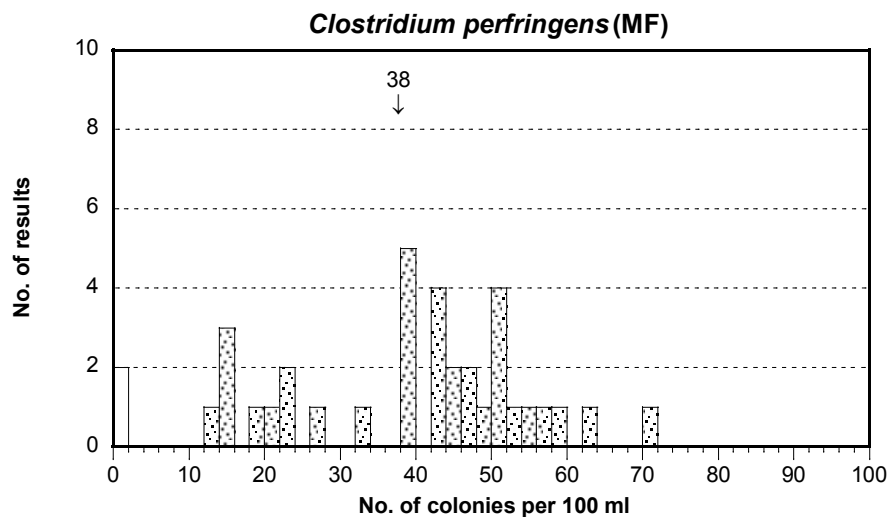
Presumptive and confirmed *Clostridium perfringens*

- The distributions looked quite well in both cases (figures 1E and F). The dispersion was small and medium in the respective analysis. There were 10 more

- presumptive results compared to confirmed. In many cases were either presumptive or confirmed results reported, sometimes both.
- 1 false negative result and 1 high outlier were reported in the presumptive analysis and 2 false negative results in the confirmed analysis.
  - No overrepresentation of low results was apparent this time as often before (5). However as before, the results (without outliers) with m-CP agar had a lower mean than those with TSC Agar also in this round(see table 10 and 11).
  - The means for presumptive and confirmed results were approximately equal.



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



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

Mould and yeast

- The distribution of mould results was very scattered. The dispersion was, however, only of medium order (figure 1G). The distribution of yeast was much better, with only a small dispersion (figure 1H).
- The yeast *H. uvarum* appears with about 10 times more colonies than the mould *C. cladosporoides*. Counting of yeasts can be done from the volumes 10 and/or 1 ml. The few mould colonies should preferentially be counted from the volume 10 ml. With only 1 ml the mould results will be very low and, accordingly, the dispersion large. This is one explanation to the scattered results of mould.

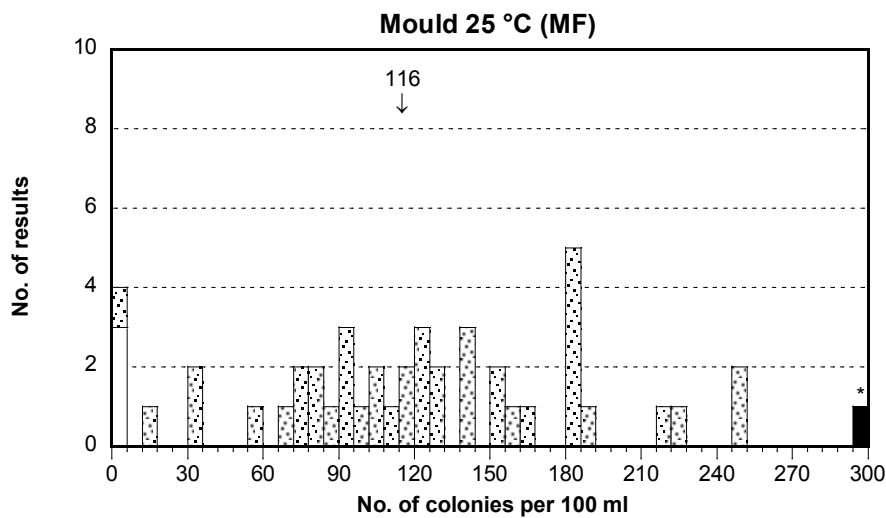


Figure 1G *Mixture A*, see figure 1A for explanations

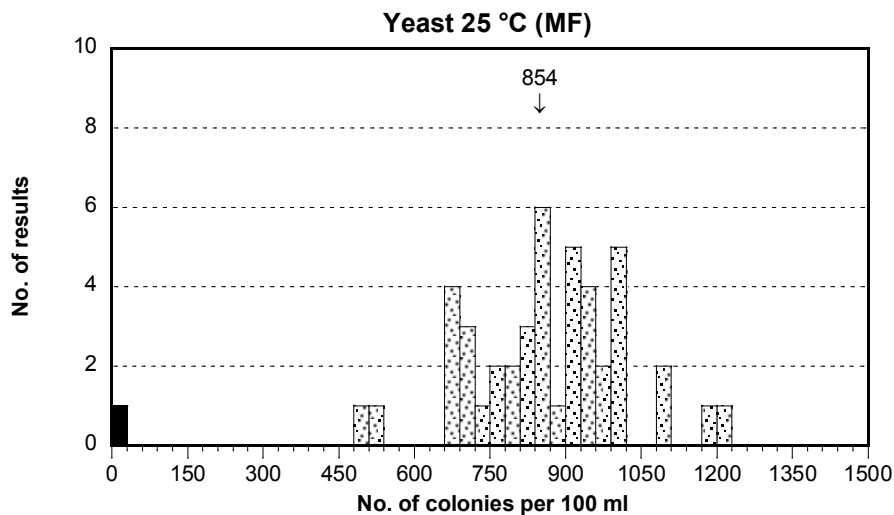


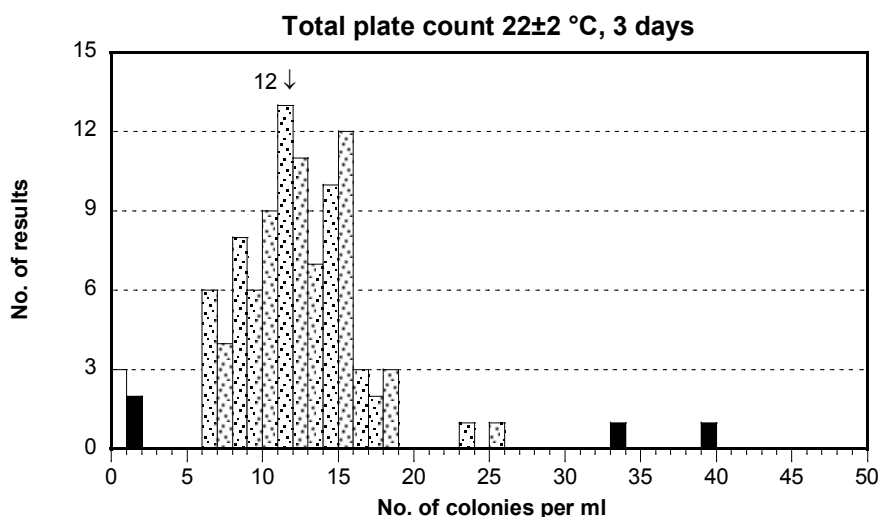
Figure 1H *Mixture A*, see figure 1A for explanations



- The yeast seems to exert some hampering effect on the mould colonies in certain volumes (e.g. 5-20 ml). Some colonies are then seen only as greenish spots in yeast colonies. This might be another explanation to the scattered mould results.

#### Culturable microorganisms

- The results were well distributed (figure 1I). The dispersion was small.
- 3 false negatives and 2 low and 2 high outliers were reported.
- Almost all detected colonies should be from the yeast *H. uvarum*. Some individual colonies of the mould as well as of *E. coli* and *E. cloacae* might also appear.



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

#### **Mixture B**

##### General information about the mixture

The mixture contained three bacterial strains and one yeast (table 1 and **table 5**): the coliform bacterium *C. freundii*, *C. perfringens*, the yeast *C. glabrata* and the bacterium *S. maltophilia*, which dominates in the analysis of culturable microorganisms. Also *C. freundii* and the yeast may emerge in that analysis.

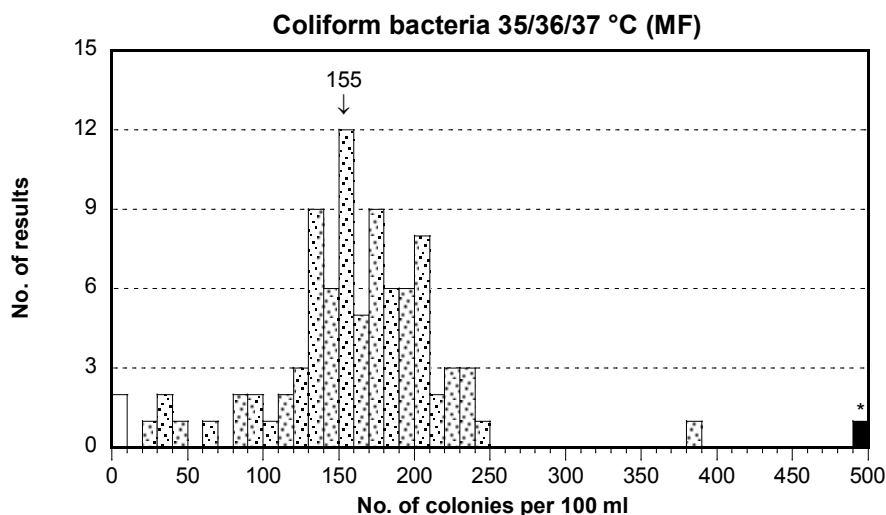
The numbers of false positive and false negative results as well as low and high outliers are reported in annex A and their relative shares in table 5.

**Table 5.** The outcome of each analysis in 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>C. freundii</i>	161	—				
Coliform bacteria (MF)	<i>C. freundii</i>	155	18	-	2	0	1
Susp. thermotol. colif. bact. (MF)	—	0	—				
<i>E. coli</i> (MF)	—	0	-	3	-	-	-
Coliform bact. (rapid method)	<i>C. freundii</i>	141	16	-	0	2	0
<i>E. coli</i> (rapid method)	—	0	-	5	-	-	-
Presumptive <i>C. perfringens</i> (MF)	<i>C. perfringens</i>	3	43	-	0	0	2
<i>C. perfringens</i> (MF)	<i>C. perfringens</i>	3	53	-	0	0	0
Mould (MF)	—	0	-	4	-	-	-
Yeast (MF)	<i>C. glabrata</i>	33	12	-	0	0	7
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>S. maltophilia</i> ( <i>C. freundii</i> ) ( <i>C. glabrata</i> )	147	9	-	0	2	2

Coliform bacteria, MF

- The results were, in principle, well distributed, but with a small over-representation of low results. The dispersion was small (figure 1J).
- 2 false negatives and 1 low outlier were reported.
- One coliform bacterium, *C. freundii*, was included in the mixture. It's colonies are relatively small and rounded on both m-Endo Agar LES and LTTC Agar at

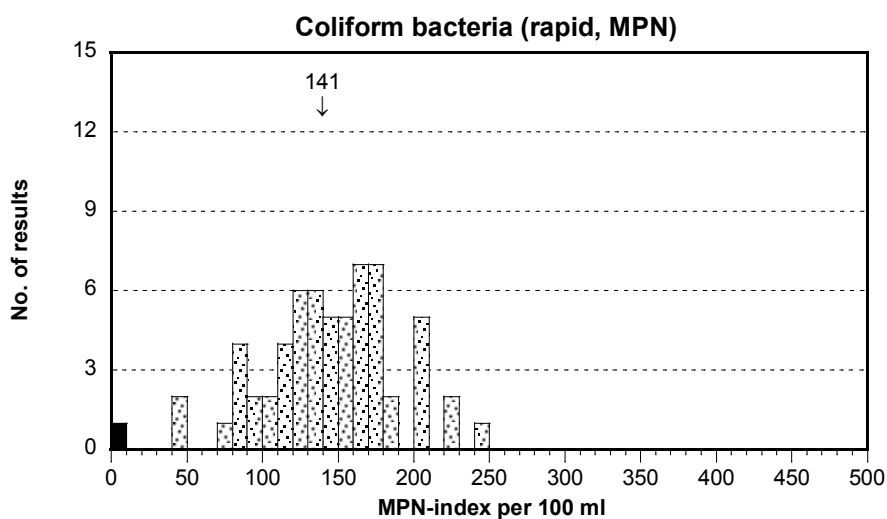


**Figure 1J** Mixture B, see figure 1A for explanations

37 °C. They show a typical metallic sheen on m-Endo Agar LES but are less typical on LTTC Agar, as they are transparent there with yellow middle.

#### Coliform bacteria, rapid method

- The distribution of results is similar to that of the MF method. The dispersion was small also here (figure 1K).
- 1 low outlier was reported.
- *C. freundii* is a coliform bacterium that possesses the enzyme  $\beta$ -galactosidase, and is therefore detected by methods based on that enzyme.



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

#### Suspected thermotolerant coliform bacteria, MF

No thermotolerant coliform bacteria were included. At a too low temperature (< 43.5 °C) small colonies of *C. freundii* may appear on m-FC Agar. Four false positive results were recorded.

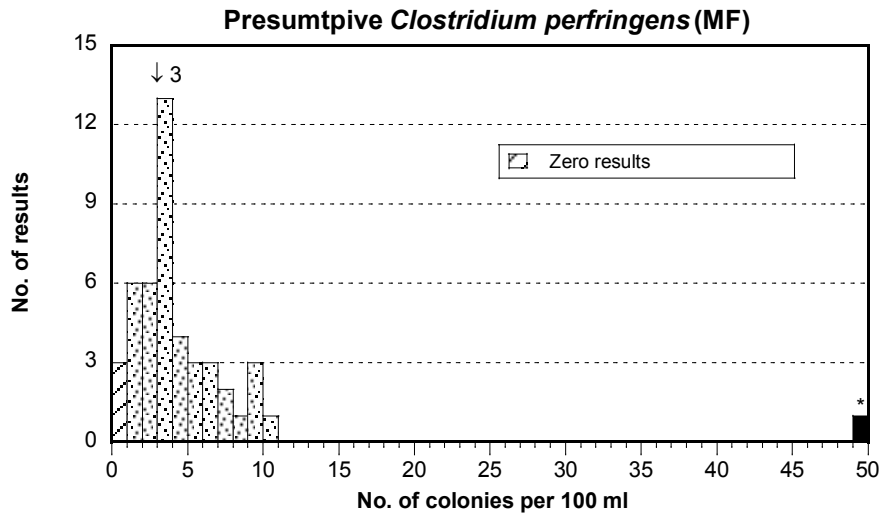
#### *E. coli*, rapid method included

- No *E. coli* was present in the mixture and a correct result should be zero. Yet, 3 false positive results were recorded in each of the MF and the rapid method.

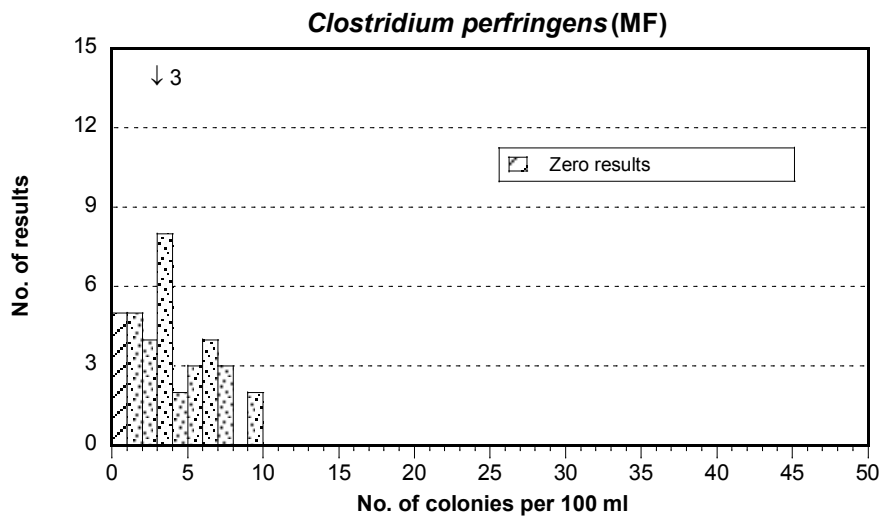
#### Presumptive and confirmed *Clostridium perfringens*

- With the low content of *C. perfringens* (3 cfu/100 ml) the distributions were good (figures 1L and M). The relative dispersion was very large in both analyses due to the low content. There were 10 more presumptive results compared to confirmed. Either presumptive or confirmed results were reported by a laboratory in many cases, but sometimes both.

- 1 high outlier was reported for the presumptive analysis. Moreover, there were a number of zero results reported, which is normal with such a low concentration.



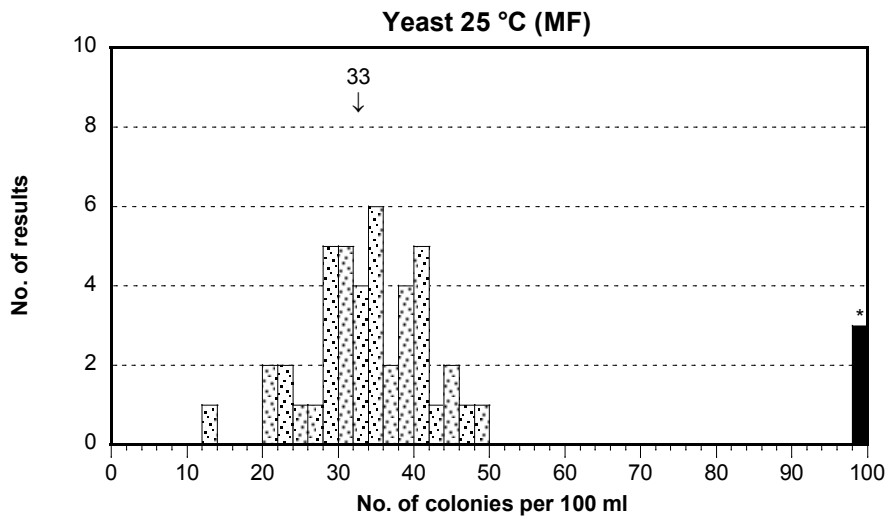
**Figure 1L** *Mixture B*, see figure 1A for explanations



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

#### Mould and yeast

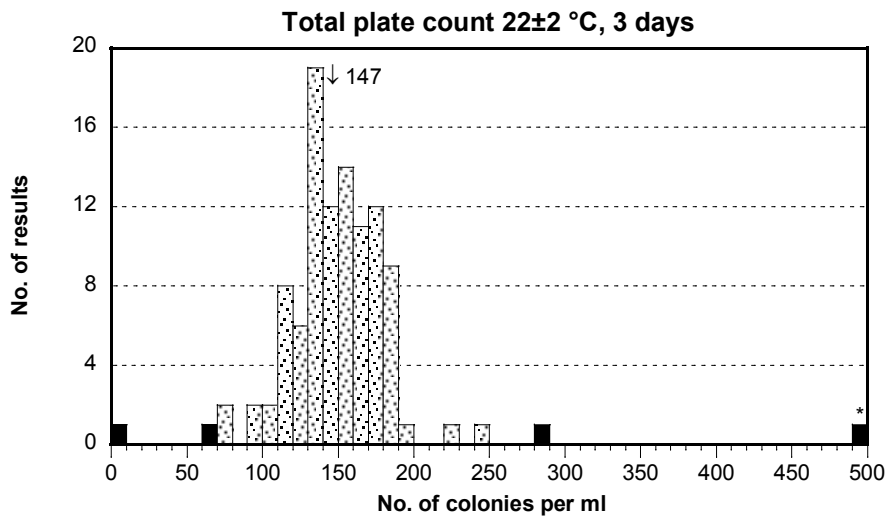
- No moulds were present in the mixture.
- The yeast results were well distributed. The dispersion was small (figure 1N).
- 3 high outliers were reported.
- *C. glabrata* grows with typical yeast colonies on relevant media. They will also emerge in the analysis of culturable microorganisms 22 °C, 3 days.



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

Culturable microorganisms

- The results were well distributed (figure 1O). The dispersion was very small.
- 2 low and 2 high outliers were reported.
- The colonies were almost totally made up of *S. maltophilia*.



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

### Mixture C

General information about the mixture

The mixture contained three bacterial strains and one mould (table 1 and **table 6**): the coliform bacteria *E. coli* and *K. oxytoca*, the mould *Ph. malorum* and the bacterium *P. fluorescens* that grows as culturable microorganism at 22 °C.

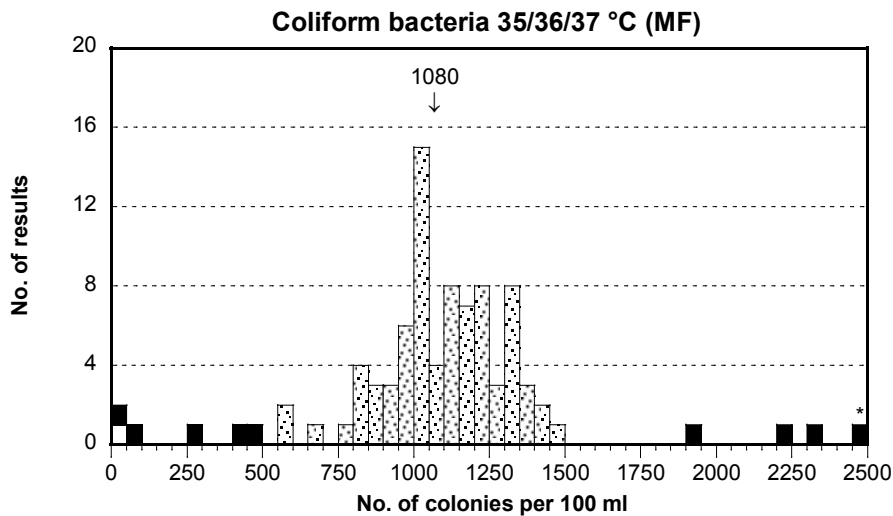
The numbers of false positive and false negative results as well as low and high outliers are reported in annex A and their relative share in table 6.

**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>E. coli</i> <i>K. oxytoca</i>	1105	—				
Coliform bacteria (MF)	<i>E. coli</i> <i>K. oxytoca</i>	1080	9	-	1	5	4
Susp. thermotol. colif. bact. (MF)	<i>E. coli</i>	480	—				
<i>E. coli</i> (MF)	<i>E. coli</i> { <i>K. oxytoca</i> }	600	19	-	1	2	0
Coliform bact. (rapid method)	<i>E. coli</i> <i>K. oxytoca</i>	1344	11	-	0	3	0
<i>E. coli</i> (rapid method)	<i>E. coli</i>	632	10	-	2	3	2
Presumptive <i>C. perfringens</i> (MF)	—	0	—	4	-	-	-
<i>C. perfringens</i> (MF)	—	0	—	6	-	-	-
Mould (MF)	<i>Ph. malorum</i>	4	60	-	0	0	2
Yeast (MF)	—	0	—	7	-	-	-
Culturable microorganisms (total count) 22±2 °C, 3 days	<i>P. fluorescens</i> <i>E. coli</i> <i>K. oxytoca</i>	25	19	-	0	2	1

#### Coliform bacteria, MF

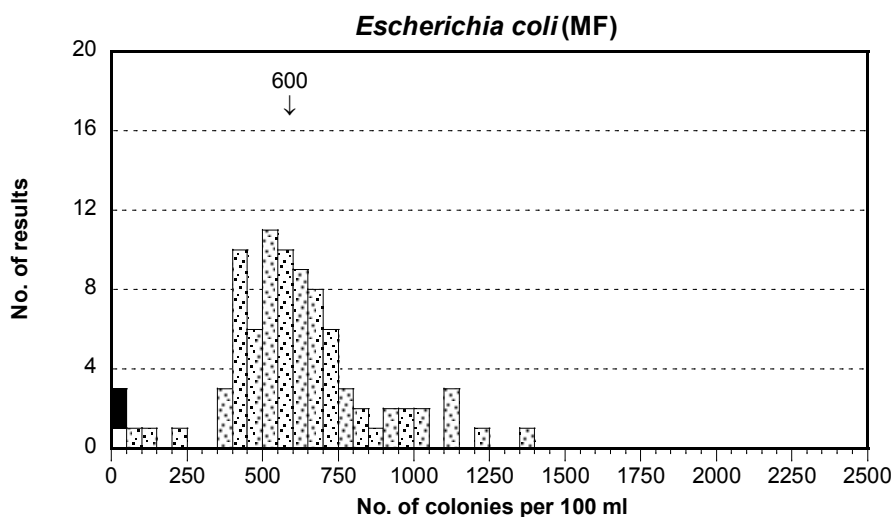
- The distribution was generally good but an unusually high number of both low and high outliers were present. The dispersion was very small (figure 1P).
- 1 false negative result and 5 low and 4 high outliers were reported.
- Both the strains of *E. coli* and *K. oxytoca* grow with typical colonies on both m-Endo Agar LES and LTTC Agar.



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

**E. coli, MF**

- The result distribution was a bit skewed to the higher end. The dispersion was small (figure 1Q) but somewhat higher than for the coliform bacteria.
- 1 false negative result and 2 low outliers were reported.
- At 44/44.5 °C only the *E. coli* strain will grow on agar media.
- At 35-37 °C both the strains of *E. coli* and *K. oxytoca* grow with typical colonies on m-Endo Agar LES and LTTC Agar. There *E. coli* has to be discerned by confirmation. When indole production is tested in broth with tryptophane, the *E. coli* confirms positive, but also the strain of *K. oxytoca* may grow there and show positive indole reaction. Test of gas production or, nowadays, test of  $\beta$ -



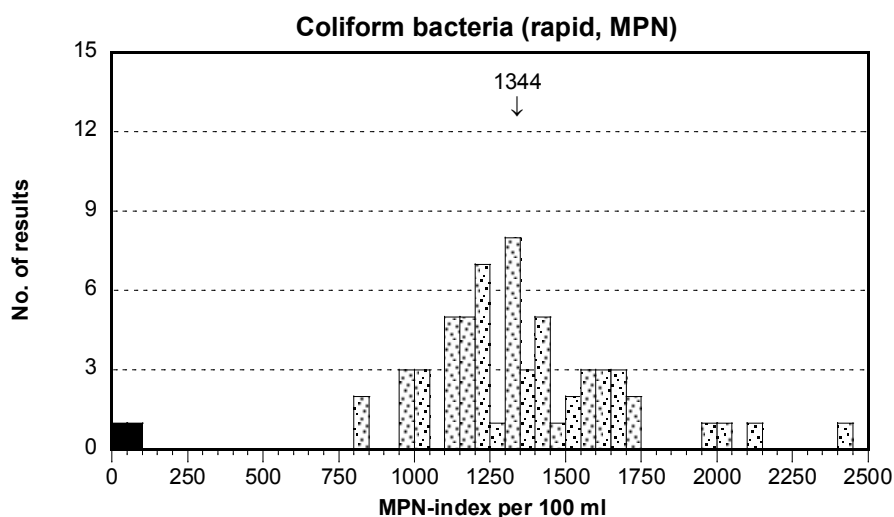
**Figure 1Q** *Mixture C*, see figure 1A for explanations

glucuronidase activity (e.g. with MUG) is sometimes used in addition to confirm *E. coli* unambiguously. In both these tests *K. oxytoca* is negative while *E. coli* is positive.

- The unexpected high counts may possibly be explained by the fact that colonies of *K. oxytoca* are interpreted as *E. coli*. Compare with the results for the rapid method below.

#### Coliform bacteria, rapid method

- The results were well distributed, with exception of some high results. The dispersion was despite that small (figure 1R).
- 2 low outliers were reported.
- Both *E. coli* and *K. oxytoca* will be regarded as coliform bacteria by methods based on activity of the enzyme  $\beta$ -galactosidase, such as Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup>.

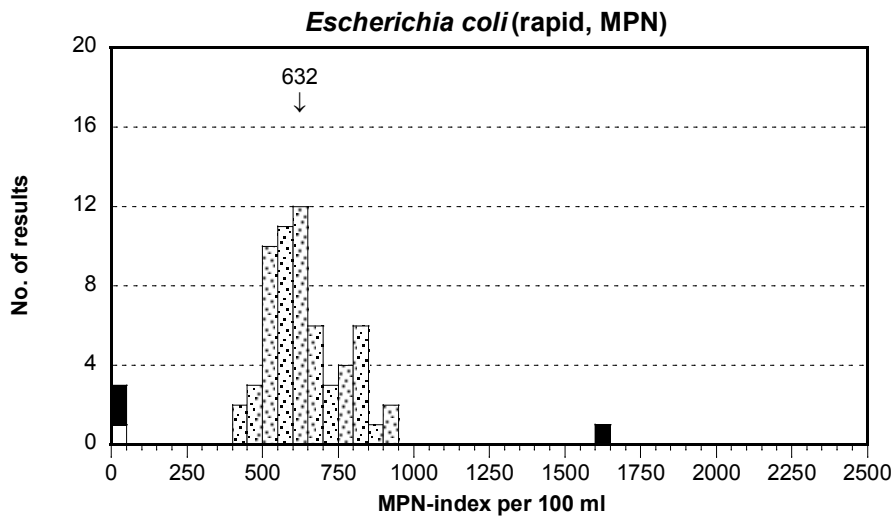


**Figure 1R** *Mixture C*, see figure 1A for explanations

#### *E. coli*, rapid method

- The results were well distributed (figure 1S). The dispersion was small.
- 1 false negative result and 2 low and 1 high outlier were reported.
- The *E. coli* strain in the mixture is  $\beta$ -glucuronidase positive, and is thus detected as *E. coli* by Colilert<sup>®</sup>-18/24 Quanti-Tray<sup>®</sup>. The strain of *K. oxytoca*, which is indole positive like *E. coli*, is however  $\beta$ -glucuronidase negative. It will not be detected as *E. coli* with e.g. that method.





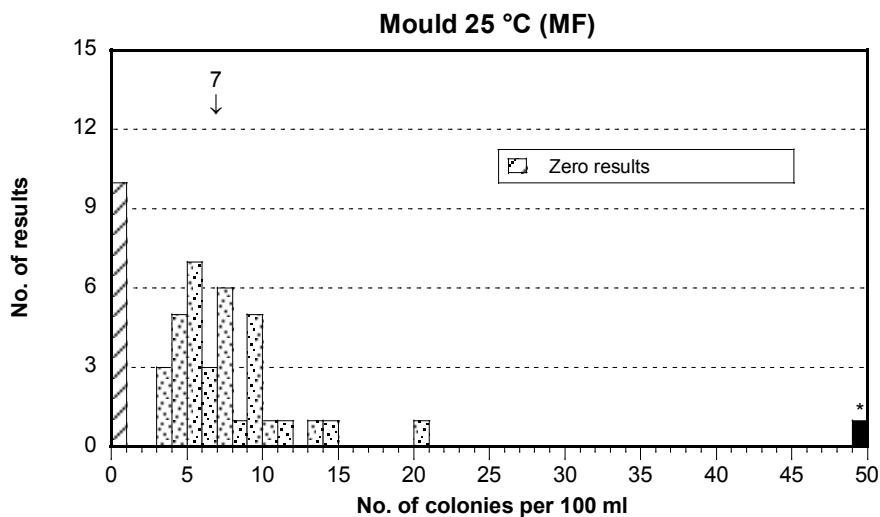
**Figure 1S** *Mixture C*, see figure 1A for explanations

**Presumptive and confirmed Clostridium perfringens**

There were no *C. perfringens* or other bacteria that could be mixed up with that species in the mixture. No false positive results were reported.

**Mould and yeast**

- There was no yeast in the mixture. Yet, 3 false positive results were present.
- One mould was included and the distribution of the results were normal with exception of some zero results (figure 1T). The average was low and the relative



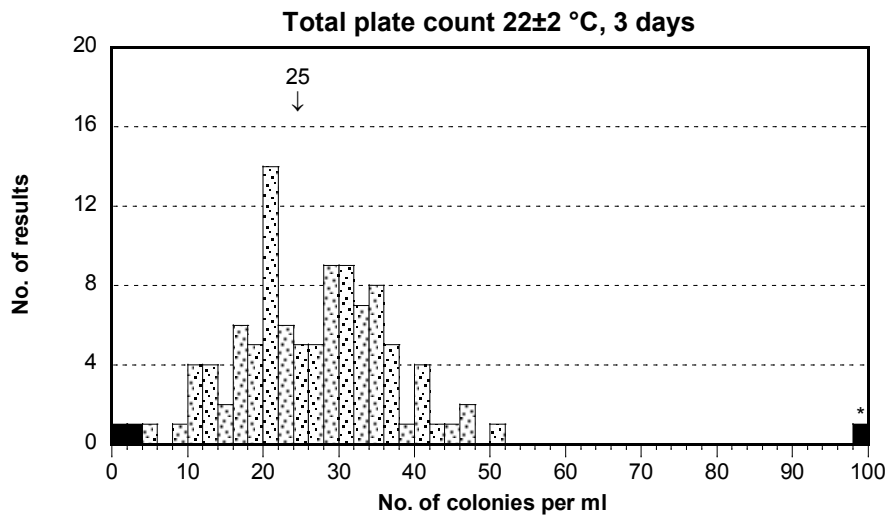
**Figure 1T** *Mixture C*, see figure 1A for explanations

dispersion therefore very large. The average was 4 cfu with zero results included and 7 cfu without.

- The 10 zero result is an overrepresentation despite the low average. The reason is not clear. The colonies could possibly be mistaken for yeast colonies because their sporulation is late. However, only 3 of the laboratories with zero results have reported any yeast colonies instead. In one of these cases, the result is even unreasonably high.

#### Culturable microorganisms

- The results were well distributed (figure 1U). The dispersion was small.
- 2 low and 1 high outlier were reported.
- The colonies are mainly made up by *P. fluorescens*. However, the coliform bacteria comprise approximately 40%.



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

## Outcome of the methods

### General information regarding methods

From this testing round onwards it is mandatory to give method information, by using the web form, for methods for which you want to report analytical results and that will be evaluated. Earlier this has been voluntary. The effect is that method information is reported for 100% of the numerical results in this round. Because of this, no table is given showing the proportion method information reported. The numbers of results for the various methods are clear from the descriptive part of **annex A**.

Method information can be recorded at any time after logging on and not necessarily in connection to current test rounds. Stated information stays in the database and need not be re-entered by the laboratory for the same method, as long as the information is valid. If method alternatives should be missing in the method forms, messages can be sent by e-mail or by use of the comment field at the bottom of the method forms. These comments are, however, only evaluated in connection to result processing and writing of the final report.

Although method information this time is available for all numerical results, they are not always easy to interpret. For some laboratories the medium stated differs from what should be the appropriate one according to the standard method stated. Results from such laboratories are not included in the compilations in the report. In other cases the interpretation must be made that one method is used for (suspected) thermotolerant coliform bacteria and another one is used for *E. coli*, where in both cases the incubation temperature has been 44/44.5 °C.

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 could result in more such deviating results than others. This might then be mentioned in the text, but for an as fair comparison as possible to be made between methods, false results and outliers are skipped. Method items with 3 or fewer results will normally not be discussed in the comparisons.

### Results for coliform bacteria and *E. coli* (MF) with different method variants

In Norway, Finland and Sweden, the national membrane filtration methods (MF) for coliform bacteria may 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"). These national methods, which are based on m-Endo Agar LES ("LES endo agar") and m-FC Agar, have usually to be used more or less modified. In Sweden and Finland, m-FC Agar must not be used for *statutory sampling* in drinking water, rather, *E. coli* should be determined by the confirmation from LES Endo Agar plates incubated at 36±2 °C. The *E. coli* confirmation in Sweden is made up by a negative oxidase test for coliform bacteria, and in addition, a positive indole test at 44 °C, and from the autumn 2010 also a positive  $\beta$ -glucuronidase

activity test. This latter test is a complement to the indole tests in order to eliminate among others, indole positive and in broth thermotolerant *K. oxytoca* strains (7). In Finland, an additional gas test at 44 °C or  $\beta$ -glucuronidase activity test is recommended as complement to the indole test. In such cases, *E. coli* should be indole positive as well as gas or  $\beta$ -glucuronidase positive.

**Table 7** Numbers of answers and results, outliers excluded, with different method standards in MF analysis of coliform bacteria (A) and *E. coli* (B) incubated at 36±2 °C

Method standard	Total no. of answ.	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><u>95</u></b>	<b><u>83</u></b>	<b><u>68</u></b>	<b><u>81</u></b>	<b><u>156</u></b>	<b><u>76</u></b>	<b><u>1082</u></b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	33	24	65	24	137	21	1026
SS 028167 <sup>b</sup>	27	25	76	24	164	24	1180
SFS 3016 <sup>c</sup>	28	27	68	26	170	24	1057
NS 4788 <sup>d</sup>	3	3	52	3	161	3	1055
Other	4	4	39	4	129	4	967
<b>B. Escherichia coli</b>	<b><u>53</u></b>	<b><u>47</u></b>	<b><u>40</u></b>	<b><u>47</u></b>	<b><u>0</u></b>	<b><u>47</u></b>	<b><u>601</u></b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	15	11	48	12	0	11	565
SS 028167 Modif. <sup>b, e</sup>	17	17	42	16	0	17	579
SFS 3016/4088 Modif. <sup>e, f, g</sup>	18	16	38	16	0	16	669
NS 4792 <sup>h</sup>	1	1	37	1	0	1	510
Other	2	2	9	2	0	2	506

1 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 from the autumn 2010 also are  $\beta$ -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  $\beta$ -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. There are also the terms SS 028167 Modif. and SFS 3016/4088 Modif., as regards *E. coli*. They involve modifications such as e.g. those that were stated above, with respect to Sweden and Finland, respectively. Individual results obtained with other methods or where the method is not known is not discussed here.

Regarding coliform bacteria, there is no general difference of significance, in the mixtures, A and C according to the **table 7A**. Different strains of *E. coli* and other coliform bacteria were present there. According to the photos in annex B the colony appearance is typical and quite easily interpreted. For the mixture B the reference method XX-EN ISO 9308-1 seems to give somewhat lower results.

**Table 8** Numbers of answers and results, with different method standards in analysis of suspected thermotolerant coliform bacteria (A; all results) and *E. coli* (B; outliers excluded) with membrane filtration and incubation at 44/44.5 °C

Method standard	Total no. of answ.	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><u>56</u></b>	<b><u>43</u></b>	<b><u>37</u></b>	<b><u>40</u></b>	<b><u>0</u></b>	<b><u>43</u></b>	<b><u>479</u></b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	11	9	39	7	0	9	567
SS 028167 <sup>b</sup>	15	10	36	10	0	10	518
SFS 4088 <sup>c</sup>	21	17	35	17	0	17	417
NS 4792 <sup>d</sup>	7	6	36	6	0	6	559
Other	2	1	100	0	—	1	100
<b>B. <i>Escherichia coli</i></b>	<b><u>11</u></b>	<b><u>11</u></b>	<b><u>41</u></b>	<b><u>11</u></b>	<b><u>0</u></b>	<b><u>11</u></b>	<b><u>605</u></b>
XX-EN ISO 9308-1:2000 <sup>a</sup>	2	2	39	2	0	2	664
SS 028167 <sup>b</sup>	1	1	41	1	0	1	456
SFS 4088 <sup>c</sup>	4	4	37	4	0	4	552
NS 4792 <sup>d</sup>	2	2	38	2	0	2	493
Other	2	2	53	2	0	2	880

1 Mean values based on square root transformation

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

Probably this is caused by the fact that the coliform bacteria are made up of a strain of *C. freundii* that gives colonies with a faint yellow colour on the LTTC medium (annex B). With a lot of colonies present, the whole agar plate turns yellow making the yellow colouration of the medium by the individual colonies difficult to discern. Furthermore, the strain of *S. maltophilia* will appear as disturbing background flora with greenish colonies. The colonies are typical for coliforms on LES endo Agar.

**Table 7B** accounts for the outcome of *E. coli* that is confirmed after primary incubation at 36±2 °C. Mixture B did not contain any *E. coli*. Mixture A contained two strains of *E. coli*, of which one is  $\beta$ -glucuronidase negative. The reference method seems to give somewhat higher values there. In mixture C with another *E. coli* strain the reference method does not give the highest average result, but the modified Finnish method instead. Different methods, thus show varying recovery with different strains.

**Table 8** accounts for the outcome of 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 are used in a greater extent than EN ISO 9308-1:2000. A slight difference exists between the methods in mixture C according to **table 8A**. The Finnish standard has there yielded lower results despite that all the results were obtained after incubation at 44 °C. There are too few results in **table 8B** to make any interpretations regarding *E. coli*.

According to **table 9** there might be slight differences in the outcome based on different primary media independent of which method standard served as base.

**Table 9** Number 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 answ.	Mixture					
		A		B		C	
		n	Mv <sup>1</sup>	n	Mv <sup>1</sup>	n	Mv <sup>1</sup>
<b>Medium</b>	<u>95</u>	<u>83</u>	<b><u>68</u></b>	<u>81</u>	<u>156</u>	<u>76</u>	<u>1082</u>
m-Endo Agar/Broth LES	64	56	71	54	166	52	1116
”LTTC Agar” <sup>2</sup>	26	24	65	24	137	21	1026
Chromocult Agar	1	1	11	1	158	1	1000
Other	3	2	39	2	115	2	844
<b>Incubation temperature</b>	<u>95</u>	<u>83</u>	<b><u>68</u></b>	<u>81</u>	<u>156</u>	<u>76</u>	<u>1082</u>
35 °C	25	23	72	22	157	22	1180
36 °C	18	16	60	15	161	14	1088
37 °C	50	42	68	42	151	38	1018
Other	2	2	71	2	189	2	1203

**Table 9** *continued*

<b>B. <i>Escherichia coli</i> MF</b>	<b>Total no. of answ.</b>	<b>Mixture</b>					
		<b>A</b>		<b>B</b>		<b>C</b>	
		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>	<b><u>53</u></b>	<b><u>47</u></b>	<b><u>40</u></b>	<b><u>47</u></b>	<b><u>0</u></b>	<b><u>47</u></b>	<b><u>601</u></b>
m-Endo Agar/Broth LES	37	34	40	33	0	34	618
”LTTC Agar” <sup>2</sup>	13	11	48	12	0	11	565
Chromocult Agar	1	1	9	1	0	1	600
Other	1	1	9	1	0	1	420
<b>Medium 44/44.5 °C<sup>4</sup></b>	<b><u>11</u></b>	<b><u>11</u></b>	<b><u>41</u></b>	<b><u>11</u></b>	<b><u>0</u></b>	<b><u>11</u></b>	<b><u>605</u></b>
m-FC Agar/Broth	8	8	40	8	0	8	532
”LTTC Agar” <sup>2</sup>	2	2	39	2	0	2	664
Other	1	1	47	1	0	1	1200
<b>Incubation temperature</b>	<b><u>90</u></b>	<b><u>83</u></b>	<b><u>41</u></b>	<b><u>83</u></b>	<b><u>0</u></b>	<b><u>83</u></b>	<b><u>600</u></b>
From 35/36/37 °C	53	48	40	48	0	48	596
From 44/44.5 °C	15	15	41	15	0	15	614
From both 36 or 44 °C	21	19	45	19	0	19	613
Other	1	1	42	1	0	1	400

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

4 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

Lactose TTC Agar yields the lowest results for coliform bacteria in mixture B (table 9A). This outcome is, of course, a result of the fact that this medium is the one given in the reference method XX-EN ISO 9308-1. Equally low results was obtained for that method when the methods were compared. No effect of the incubation temperatures was visible.

For *E. coli*, there was no clear tendency in relation to the media (table 9B). This time there is even no indication of lower recovery for *E. coli* when the media are incubated at 44/44.5 °C compared to at 36 ± 2 °C.

### Results for *Clostridium perfringens* with different method variants

The analysis of *Clostridium perfringens* is performed in different ways in different countries and laboratories. This is probably 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 are spores and vegetative cells of *C. perfringens*. At the time when that was decided, there was no international standard for water analyses. Therefore, one method was explicitly stated in the drinking water directive, namely usage of m-CP Agar at 44 °C. It 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 processed in the standardisation work. At that time, the method was available as a Committee Draft (CD). An approval of using the most current standard draft was given by the group concerned under the EU commission. The current version at the time was ISO/CD 6461-2:2002-12-20. Since then, no new drafts have been published. Certain changes or additional paragraphs to the draft mentioned have been decided upon later at ISO standardisation meetings. These decisions may also be used and should have been conveyed by the representatives of the national standardisation bodies. This information has also been conveyed to participating laboratories by means of PT round instructions from the National Food Administration.

Another method that has been used is the older method for analysis of sulphite reducing clostridia, EN ISO 26461-2:1993. It may have been used as it is, with or without heating of the sample, or after a modification which makes it comparable to

**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 B

Method/"Standard"	Total no. of answ.	Mixture					
		A (pres. <sup>1)</sup> )		A (conf. <sup>1)</sup> )		B (pres. <sup>1)</sup> )	
		n	Mv <sup>2</sup>	n	Mv <sup>2</sup>	n	Mv <sup>2</sup>
<b><i>With stated method, total</i></b>	<b><u>57</u></b>	<b><u>44</u></b>	<b><u>42</u></b>	<b><u>34</u></b>	<b><u>38</u></b>	<b><u>45</u></b>	<b><u>3</u></b>
EN ISO 26461-2:1993 <sup>3</sup>	8	6	51	5	48	6	4
ISO/CD 6461-2:2002 <sup>4</sup>	27	25	43	9	44	26	4
EU directive (m-CP Agar) <sup>5</sup>	13	8	33	13	33	8	1
DS 2256 <sup>6</sup>	2	1	42	2	25	1	3
Other	1	1	52	0	–	1	2
<i>Unknown</i>	6	3	38	5	36	3	4

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

2 Mean values calculated based on square root transformation

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, Bestämmelse af *Clostridium perfringens*, 1 udg., Jan 1983



ISO/CD 6461-2:2002. Such a modification is e.g. the introduction of confirmation steps.

It is in many cases unclear exactly how the methods have been used. It is clear from **table 10** that the mean values for the laboratories that reported presumptive results are lower in mixture A as well as in mixture B when m-CP Agar was used, in comparison to the two other methods used with more than 3 results. This is in agreement with the results reported in spring 2008 (5). In this round also the confirmed results follow the same trend, though they are shown only for mixture A. The confirmed results in mixture B are equally low as the presumptive ones making the absolute differences between methods very small.

When using m-CP Agar, there are no special presumptive results really, but they ought to be identical with 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. In mixture B the average for 13 laboratories stating the use of m-CP Agar was 2 cfu/100 ml for (confirmed) *C. perfringens*. The total average for all methods was there 3 cfu/100 ml.

From table 10 it is clear that laboratories that have used EN ISO 26461-21993 in any way have obtained the highest average, at least in mixture A.

**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 B

Method variant	Total no. of answ.	Mixture					
		A (pres. <sup>1</sup> )		A (conf. <sup>1</sup> )		B (pres. <sup>1</sup> )	
		n	Mv <sup>2</sup>	n	Mv <sup>2</sup>	n	Mv <sup>2</sup>
<b><i>Substrate</i></b>	<b><u>57</u></b>	<b><u>44</u></b>	<b><u>42</u></b>	<b><u>34</u></b>	<b><u>38</u></b>	<b><u>45</u></b>	<b><u>3</u></b>
“PAB/TSC Agar” 44 °C <sup>3</sup>	37	32	44	16	45	33	4
“SFP Agar” <sup>4</sup>	2	1	49	1	49	1	9
m-CP Agar <sup>5</sup>	15	9	34	15	32	9	1
Iron Sulphate Agar <sup>6</sup>	2	1	42	2	25	1	3
Other	1	1	46	0	–	1	3
<i>Unknown</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 & Kanamycine.

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

6 No specific antibiotic is included in Iron Sulphate Agar.

In total 25 out of 57 laboratories have given results for both presumptive and confirmed *C. perfringens*. Thus, it is only partly the same laboratories that have reported both presumptive and confirmed results. This explains the apparently lower results for confirmed compared to presumptive *C. perfringens* in mixture A.

The results obtained with different media and to some extent other conditions, regardless of which method was stated by the laboratories, are made clear in **table 11**. As above, considerably lower results can be observed with respect to m-CP Agar, in particular in mixture A.

All accepted analytical results were obtained after anaerobic incubation, most of them after incubation at 44 °C.

## The outcome of deviating results – judgement

The reported results from all laboratories are accounted for in **annex A**. A summary of the results from each laboratory in annex A – apart from false results – is given by a box plot in **figure 2**. The lesser range from smallest to largest value, and the more centred around the standard value zero, the greater is the similarity among the laboratory results *and those averages obtained by pooling all accepted laboratory results*.

The laboratories are not grouped or ranked based on their results. The **judgement** that is made **aims to** clearly give information regarding the **number of false results and outliers**. These are presented in the tables below the box plots. Besides, false results and outliers are shaded and put in boldface in annex A. There are also limits for lowest and highest value accepted in each analysis stated in the summarizing rows at the bottom of annex A.

Annex C with z-values are not commented or evaluated specifically here. They are the base for the box plots. They are mainly given to simplify the follow-up process for those laboratories that want to use z-values.

In cases where it is obvious, it is also stated if a laboratory has mixed up the analytical results. If whole mixtures have been mixed up, it is shown by crossing out the sample numbers in question in annex A.

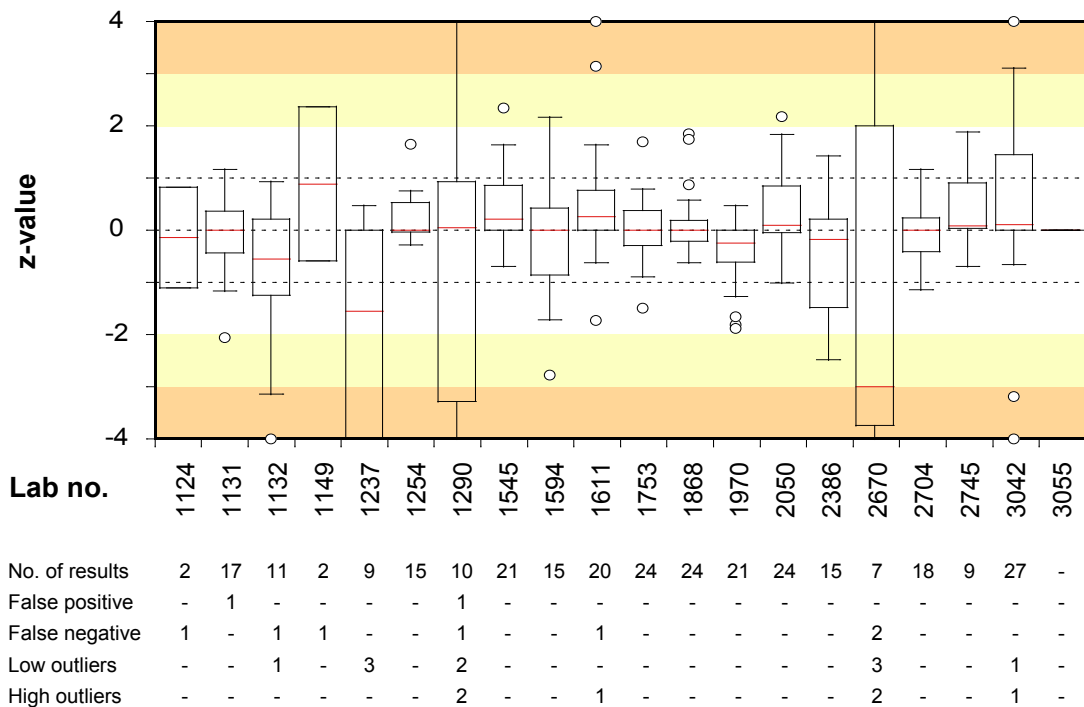
Laboratories that have not reported their results have to compare their results themselves with the corresponding ones from other laboratories in annex A.

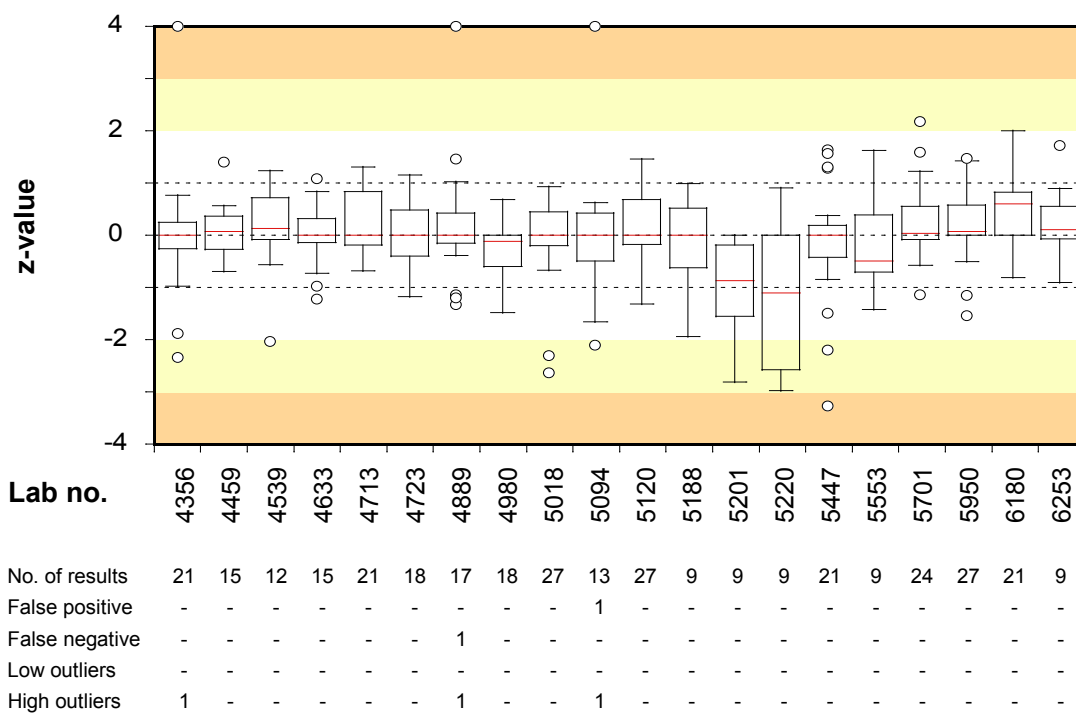
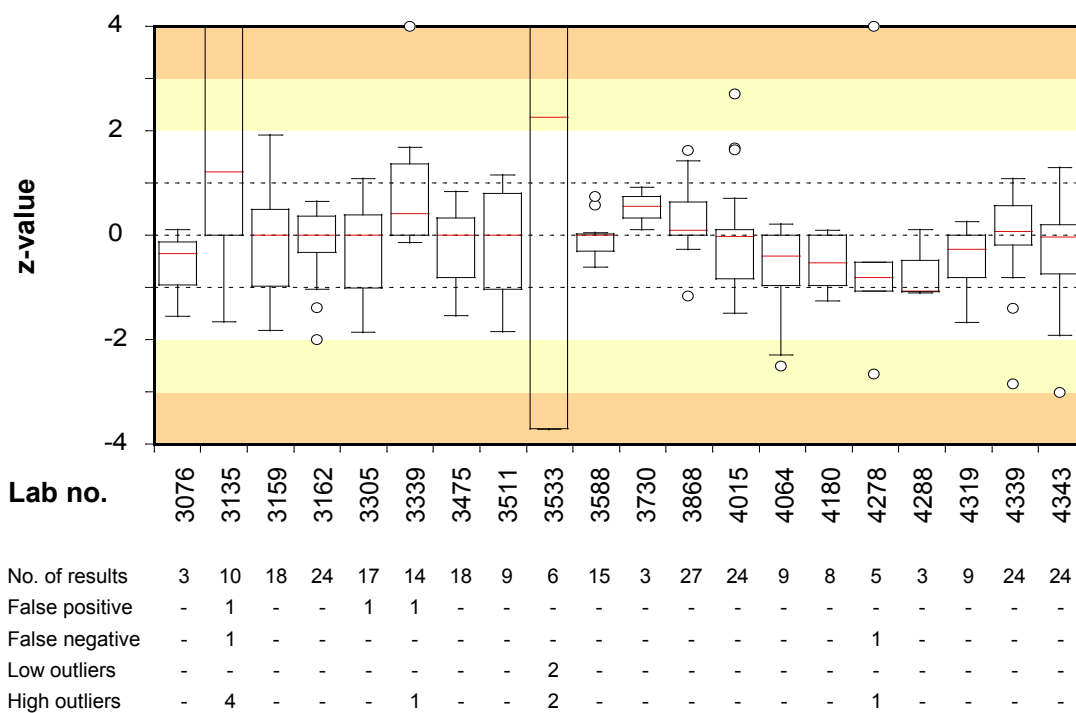
For description of how the analytical results have been treated and for recommendations on how follow-up of the results may be done, please see the scheme protocol (3). It is found as a PDF document on the website of our schemes [www.slv.se/absint](http://www.slv.se/absint).

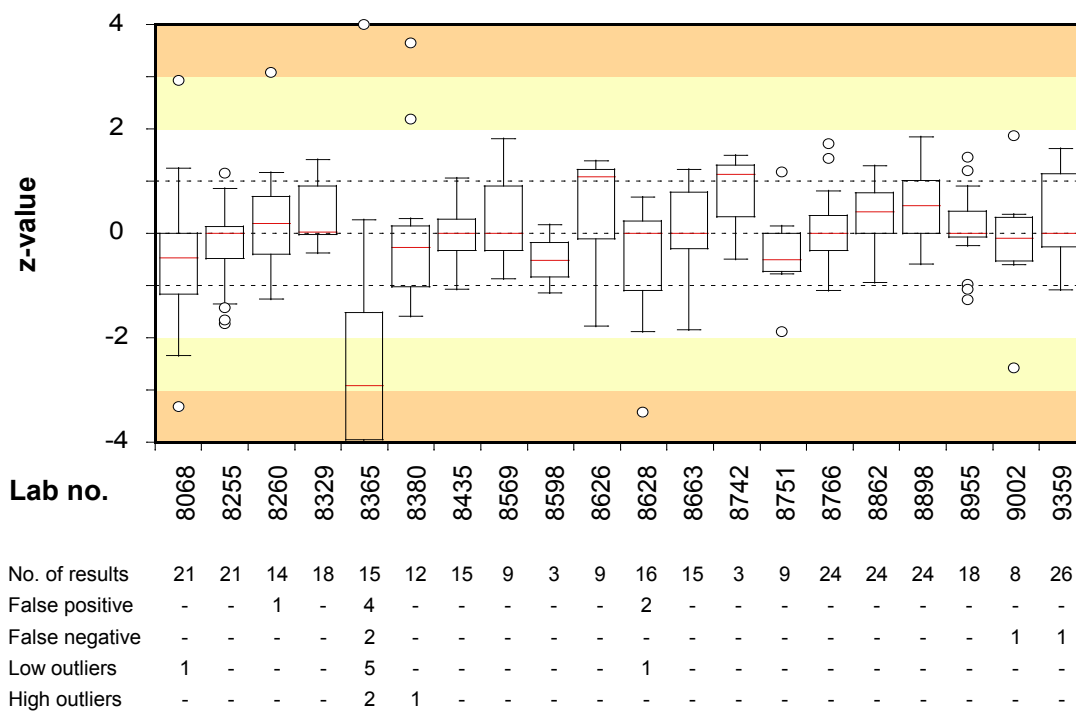
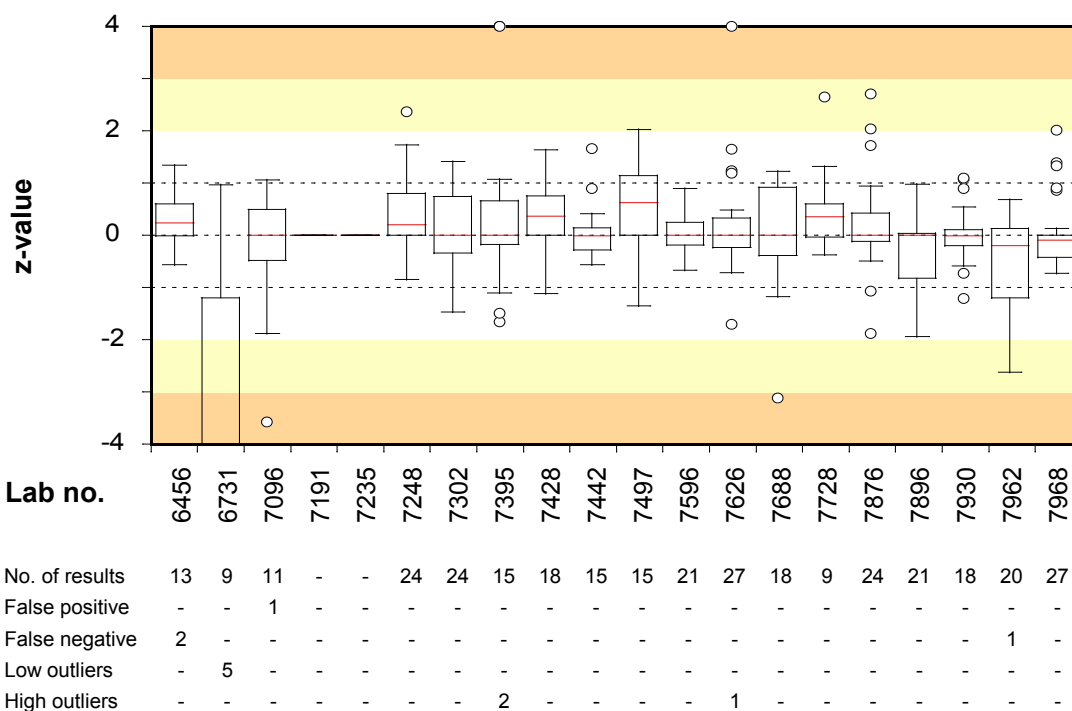
**Figure 2** Box plots and number of deviating values for each participating laboratory. The square root transformed results of a laboratory is converted into standardised values (z-value) to be able to compare the different analyses.

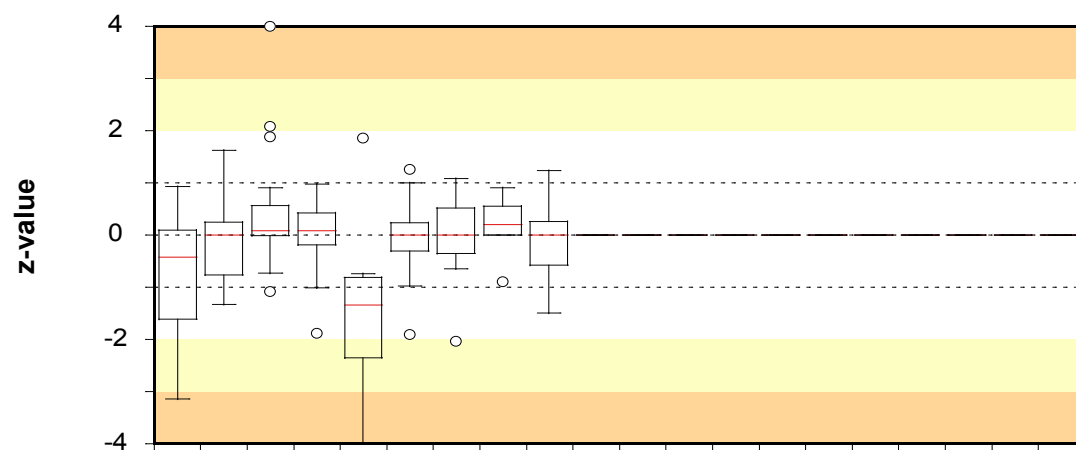
- Standardised values are calculated from the formula  $z = (x - mv) / s$
- Standardised values  $> +4$  and  $< -4$  have in the plots received the values  $+4$  and  $-4$ , respectively.
- False results do not generate z values and are not included in 'No. of results'. False positive results cannot 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  $> [\text{largest value of the box} + 1.5 \times (\text{largest value of the box} - \text{smallest value of the box})]$









Lab no.	9436	9451	9465	9569	9655	9736	9897	9899	9903
No. of results	27	15	15	27	8	24	15	18	18
False positive	-	-	-	-	1	-	-	-	-
False negative	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	1	-	-	-	-
High outliers	-	-	1	-	-	-	-	-	-

## References

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**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

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
1124	1 3 2	95	630	740	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1131	1 3 2	100	210	910	100	210	910	-	-	-	67	<b>73</b>	630	70	126	810	14	0	500
1132	3 2 1	64	0	900	32	<b>0</b>	<b>445</b>	-	-	-	50	0	436	-	-	-	15	0	750
1149	2 1 3	83	154	1120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1237	1 2 3	-	-	-	80	33	800	-	-	-	50	0	500	-	-	-	-	-	-
1254	1 3 2	-	-	-	84	200	1100	40	0	540	40	0	540	-	-	-	-	-	-
1290	2 1 3	-	-	-	<b>730</b>	60	<b>46</b>	-	-	-	<b>730</b>	0	<b>28</b>	-	-	-	-	-	-
1545	1 2 3	101	167	1380	101	167	1380	101	0	650	101	0	650	-	-	-	-	-	-
1594	3 2 1	32	190	1100	32	190	1100	11	0	5	19	0	1100	33	152	1986	13	0	921
1611	3 2 1	76	170	2200	76	170	<b>2200</b>	42	0	440	38	0	1027	80	75	2420	21	0	816
1753	3 2 1	87	172	1432	87	172	1432	-	-	-	51	0	591	80	178	1520	19	0	558
1868	3 2 1	68	123	1195	68	123	1195	-	-	-	36	0	598	75	125	1414	19	0	649
1970	2 1 3	77	180	1000	77	180	1000	42	0	470	42	0	470	-	-	-	-	-	-
2050	3 1 2	-	-	-	65	218	1464	-	-	-	38	0	745	82	177	1695	18	0	740
2386	2 1 3	56	180	1020	56	180	1020	-	-	-	56	0	760	-	-	-	-	-	-
2670	2 3 1	1	34	280	<b>1</b>	34	<b>280</b>	-	-	-	<b>1</b>	0	<b>0</b>	-	-	-	-	-	-
2704	1 2 3	-	-	-	56	180	940	-	-	-	37	0	510	83	145	1445	19	<1	624
2745	2 1 3	70	159	1260	70	159	1260	42	0	1260	42	0	1102	-	-	-	-	-	-
3042	1 3 2	153	145	990	153	145	990	68	0	990	68	0	990	<b>180</b>	200	2000	40	0	830
3055	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3076	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3135	3 1 2	-	-	-	-	-	-	-	-	-	-	-	-	<b>350</b>	220	1600	<b>70</b>	<b>2</b>	<b>1600</b>
3159	3 1 2	-	-	-	72	100	890	-	-	-	42	0	400	88.5	200.5	1445	16.4	<1	885
3162	3 2 1	73	139	1036	73	139	1036	-	-	-	11	0	389	82	126	1450	23	0	595
3305	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	91	130	1500	18	<1	680
3339	3 2 1	80	240	1150	80	240	1150	-	-	-	48	0	570	-	-	-	-	-	-
3475	1 2 3	-	-	-	90	127	1082	-	-	-	48	0	673	69	96	1120	12	<1	461
3511	2 3 1	-	-	-	-	-	-	-	-	-	-	-	-	95	99	1184	25	<1	429
3533	3 1 2	-	-	-	<b>490</b>	380	<b>480</b>	100	<b>100</b>	100	-	-	-	-	-	-	-	-	-
3588	3 1 2	83	156	1090	83	156	1090	34	0	470	34	0	470	-	-	-	-	-	-
3730	2 1 3	50	150	400	-	-	-	33	0	400	-	-	-	-	-	-	-	-	-
3868	3 2 1	86	200	1200	86	200	1200	34	0	330	43	0	550	109	144	1650	31	0	500
4015	2 3 1	45	84	1014	45	84	1014	9	0	373	17	0	423	72	150	1120	11	0	548
4064	1 2 3	-	-	-	46	134	650	-	-	-	38	<1	650	-	-	-	-	-	-
4180	3 2 1	-	-	-	68	95	995	-	-	-	43	0	470	-	-	-	-	-	-
4278	2 1 3	-	-	-	18	<b>0</b>	<b>99999</b>	-	-	-	-	-	-	-	-	-	-	-	-
4288	2 1 3	86	140	1300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4319	1 2 3	67	170	780	67	170	780	35	0	390	36	0	432	-	-	-	-	-	-
4339	2 3 1	-	-	-	82	138	1300	50	<1	600	4	<1	750	84	86	1120	17	<1	649
4343	1 2 3	59	118	1218	59	118	1218	-	-	-	3	0	545	45	110	1328	8	0	798
4356	2 1 3	72	9800	1200	72	<b>9800</b>	1200	-	-	-	58	0	620	73	130	1300	24	0	520
4459	2 1 3	-	-	-	74	182	1045	-	-	-	74	0	523	86	167	1387	21	0	562
4539	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	89	201	1184	18	0	624
4633	2 3 1	-	-	-	79	205	1300	33	0	527	39	0	650	56	101	1375	14	0	613
4713	3 2 1	76	120	1300	76	120	1300	38	0	480	48	0	500	73	120	1600	25	0	610
4723	3 1 2	73	209	1207	73	209	1207	16	0	455	21	0	604	-	-	-	-	-	-
4889	3 1 2	-	-	-	39	180	1100	-	-	-	39	<1	660	100	160	1300	16	<1	820
4980	1 2 3	42	150	980	42	150	980	30	0	440	30	0	440	78.2	83.1	1184	13.7	<1	591
5018	3 2 1	75	190	1100	75	190	1100	-	-	-	8	0	550	70	133	1223	15	0	554
5094	2 3 1	-	-	-	79	98	1000	42	0	700	42	0	700	-	-	-	-	-	-
5120	1 3 2	94	150	970	94	150	970	40	0	480	68	0	970	74	160	1300	22	0	820
5188	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	89	164	1650	25	<1	560
5201	1 2 3	16	141	995	16	130	800	-	-	-	9	0	420	-	-	-	-	-	-
5220	1 2 3	77	46	580	77	46	580	30	0	140	30	0	140	-	-	-	-	-	-
5447	2 3 1	11	158	1000	11	158	1000	-	-	-	9	0	600	-	-	-	-	-	-
5553	1 2 3	-	-	-	54	119	822	-	-	-	32	0	400	-	-	-	-	-	-
5701	3 1 2	96	166	1136	96	166	1136	-	-	-	96	<1	909	113	172	1300	19	<1	687
5950	2 3 1	109	236	1164	109	236	1164	-	-	-	62	0	591	88	172	1203	22	0	687
6180	2 3 1	90	203	1220	90	203	1220	41	0	470	54	0	610	123	168	1180	33	0	620
6253	1 2 3	-	-	-	-	-	-	-	-	-	-	-	-	77	227	1618	13	0	686
6456	2 1 3	-	-	-	87	130	1200	-	-	-	46	0	665	78	132	1184	<b>0</b>	<b>0</b>	<b>0</b>
6731	3 2 1	-	-	-	-	-	-	-	-	-	-	-	-	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>1</b>
7096	1 2 3	81	154	1150	81	154	1150	26	0	580	65	0	64	-	-	-	-	-	-
7191	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7235	2 1 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7248	1 2 3	71	177	1054	71	177	1054	29	<b>&lt;0</b>	518	26	<b>&lt;0</b>	518	93	185	2122	25	<b>&lt;0</b>	841
7302	1 2 3	78	230	1120	78	230	1120	-	-	-	65	<1	470	72	172	1240	10	<1	780
7395	1 2 3	65	220	2300	65	220	<b>2300</b>	-	-	-	60	0	610	-	-	-	-	-	-
7428	2 3 1	43	200	1170	43	200	1170	-	-	-	26	<1	520	-	-	-	30	<1	600
Mean					<b>68</b>	<b>155</b>	<b>1080</b>				<b>41</b>	<b>0</b>	<b>600</b>	<b>78</b>	<b>141</b>	<b>1344</b>	<b>19</b>	<b>0</b>	<b>632</b>
CV (%)					18	18	9				24	-	19	13	16	11	18	-	10



are mixed up. False positive and false negative values are excluded, as well as other outliers, in the summarizing calculated results at the end of the 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.

$$z = (x - mv) / s.$$

Presumptive C. perfringens (MF)			C. perfringens (MF)			Mould (MF)			Yeast (MF)			Total plate count 22 °C, 3 days			Total plate count 36±2 °C, 2 days			Lab no.
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
-	-	-	-	-	-	-	-	-	-	-	-	<1	118	34			1124	
40	3	0	-	-	-	-	-	-	-	-	-	14	151	29			1131	
-	-	-	-	-	-	-	-	-	-	-	-	14	129	4			1132	
-	-	-	-	-	-	-	-	-	-	-	-	<1	220	20			1149	
-	-	-	-	-	-	-	-	-	-	-	-	1	7	1			1237	
-	-	-	-	-	-	250	0	4	810	31	0	12	160	32			1254	
-	-	-	0	6	41	-	-	-	-	-	-	12	70	35			1290	
50	9	0	38	5	0	120	0	7	1100	40	0	14	151	19			1545	
-	-	-	-	-	-	-	-	-	-	-	-	9	147	22			1594	
-	-	-	-	-	-	0	0	6	760	38	0	15	139	28			1611	
30	2	0	-	-	-	94	0	5	964	31	0	7	125	26			1753	
49	10	0	-	-	-	180	0	3	780	48	0	11	143	21			1868	
20	2	0	20	2	0	86	0	0	930	24	0	6	150	23			1970	
47	1	0	-	-	-	110	0	4	991	41	0	13	141	51			2050	
14	0	0	14	0	0	-	-	-	-	-	-	8	142	41			2386	
-	-	-	-	-	-	-	-	-	-	-	-	0	2E+05	2E+05			2670	
-	-	-	42	7	0	-	-	-	-	-	-	8	136	16			2704	
-	-	-	-	-	-	-	-	-	-	-	-	15	175	19			2745	
42	3	0	42	3	0	1	0	9	7	28	0	25	188	30			3042	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			3055	
-	-	-	-	-	-	0	0	0	700	100	0	12	107	22			3076	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			3135	
-	-	-	14	1	0	-	-	-	-	-	-	14	120	36			3159	
38	5	0	-	-	-	59	0	6	927	23	0	14	137	31			3162	
31	1	<1	-	-	-	30	35	<1	670	20	<1	13	160	37			3305	
140	3	510	70	3	0	-	-	-	-	-	-	15	190	36			3339	
-	-	-	23	4	0	-	-	-	-	-	-	14	152	33			3475	
-	-	-	-	-	-	-	-	-	-	-	-	16	157	12			3511	
-	-	-	-	-	-	-	-	-	-	-	-	33	62	40			3533	
-	-	-	-	-	-	102	0	3	860	29	0	11	136	33			3588	
-	-	-	-	-	-	-	-	-	-	-	-	12	163	35			3730	
38	3	0	38	3	0	130	0	7	1000	38	0	14	160	25			3868	
69	3	0	-	-	-	167	0	4	887	47	0	23	116	20			4015	
-	-	-	-	-	-	-	-	-	-	-	-	12	90	21			4064	
-	-	-	-	-	-	-	-	-	-	-	-	-	114	19			4180	
-	-	-	-	-	-	-	-	-	-	-	-	9	133	16			4278	
-	-	-	-	-	-	-	-	-	-	-	-	12	118	16			4288	
-	-	-	-	-	-	-	-	-	-	-	-	9	137	27			4319	
-	-	-	51	6	<1	150	<1	7	950	28	<1	13	143	33			4339	
29	1	0	-	-	-	73	0	5	990	35	0	14	153	21			4343	
42	0	0	42	0	0	-	-	-	-	-	-	12	130	33			4356	
-	-	-	-	-	-	-	-	-	-	-	-	10	149	19			4459	
-	-	-	12	7	0	-	-	-	-	-	-	15	159	28			4539	
-	-	-	-	-	-	-	-	-	-	-	-	12	145	31			4633	
-	-	-	-	-	-	190	0	10	840	44	0	11	130	28			4713	
49	3	0	-	-	-	91	0	7	700	26	0	10	181	25			4723	
<1	120	<1	-	-	-	-	-	-	-	-	-	8	170	15			4889	
-	-	-	46	5	0	-	-	-	-	-	-	12	154	24			4980	
56	4	0	50	4	0	150	0	7	490	32	0	15	174	29			5018	
-	-	-	-	-	-	160	0	0	-	12600	700	10	148	9			5094	
51	2	0	51	2	0	180	0	9	660	31	0	11	160	31			5120	
-	-	-	-	-	-	-	-	-	-	-	-	9	140	10			5188	
-	-	-	-	-	-	-	-	-	-	-	-	11	146	13			5201	
-	-	-	-	-	-	-	-	-	-	-	-	15	118	12			5220	
62	9	0	62	9	0	70	0	5	727	36	0	7	139	22			5447	
-	-	-	-	-	-	-	-	-	-	-	-	18	158	39			5553	
52	2	<1	-	-	-	81	<1	4	910	35	<1	8	136	21			5701	
39	3	0	39	3	0	120	0	6	682	22	0	17	186	41			5950	
51	6	0	51	6	0	-	-	-	-	-	-	9	156	34			6180	
-	-	-	-	-	-	-	-	-	-	-	-	10	150	31			6253	
-	-	-	-	-	-	-	-	-	-	-	-	13	174	40			6456	
-	-	-	-	-	-	-	-	-	-	-	-	6	175	15			6731	
-	-	-	55	0	1	-	-	-	-	-	-	10	161	21			7096	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			7191	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			7235	
32	4	<0	-	-	-	106	<0	7	900	33	<0	14	177	45			7248	
43	8	<1	-	-	-	100	<1	9	940	30	<1	8	181	22			7302	
-	-	-	-	-	-	130	0	0	690	280	0	7	160	23			7395	
49	9	<1	49	9	<1	-	-	-	-	-	-	13	175	29			7428	
42	3	0	38	3	0	116	0	4	854	33	0	12	147	25			Mean	
17	43	-	21	53	-	28	-	60	9	12	-	15	9	19			CV (%)	



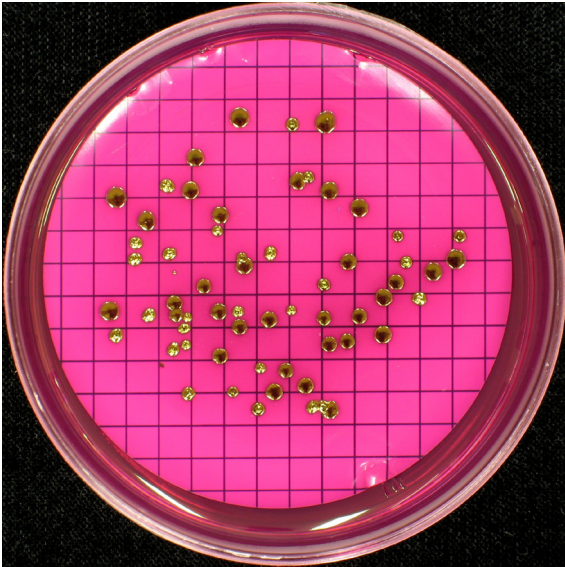


## **Annex B**

*Photographs from relevant volumes of the media for the various parameters in the mixtures*

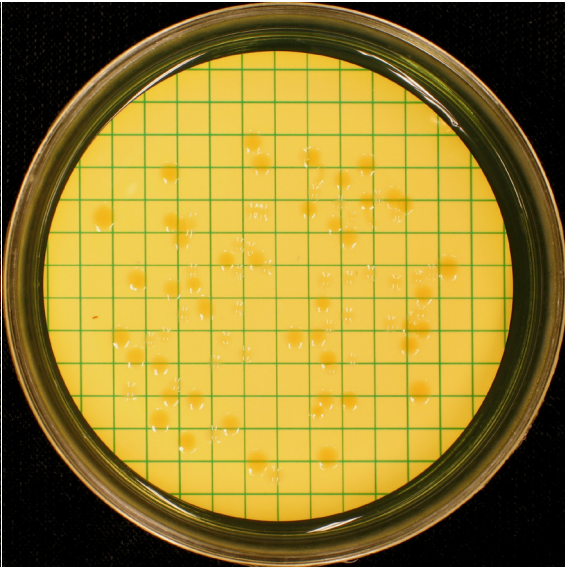
Mixture A

m-Endo Agar LES, 37 °C



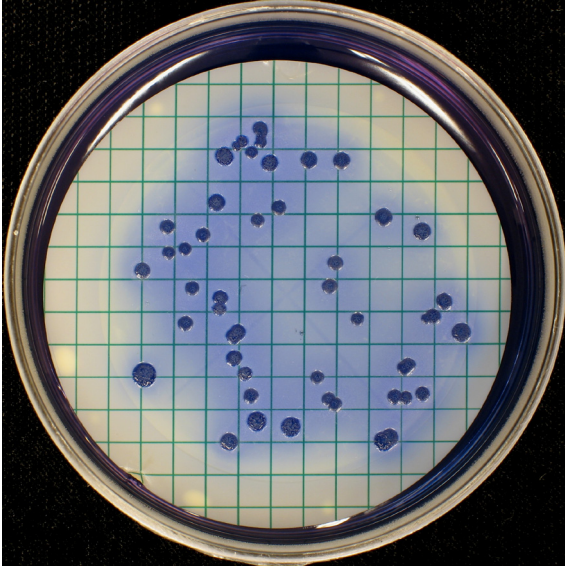
100 ml

m-Lactose TTC Agar, 37 °C



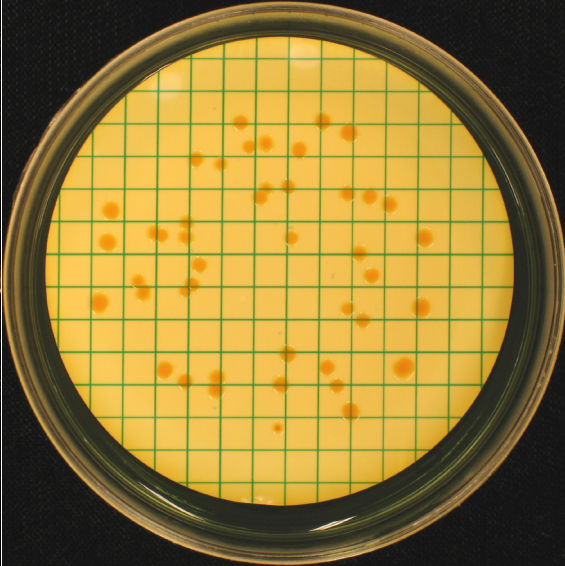
100 ml

m-FC Agar, 44 °C



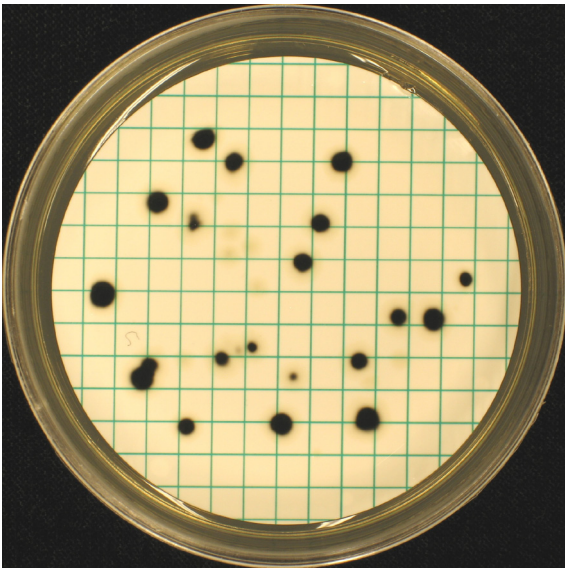
100 ml

m-Lactose TTC Agar, 44 °C



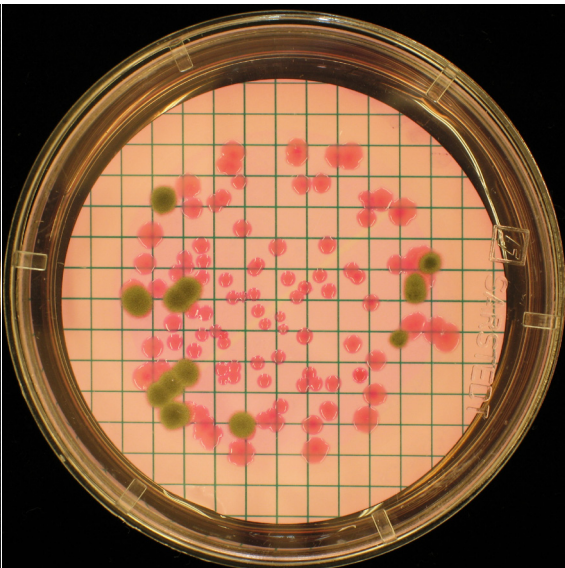
100 ml

m-TSC Agar, 44 °C



100 ml

m-Burman Agar, 25 °C

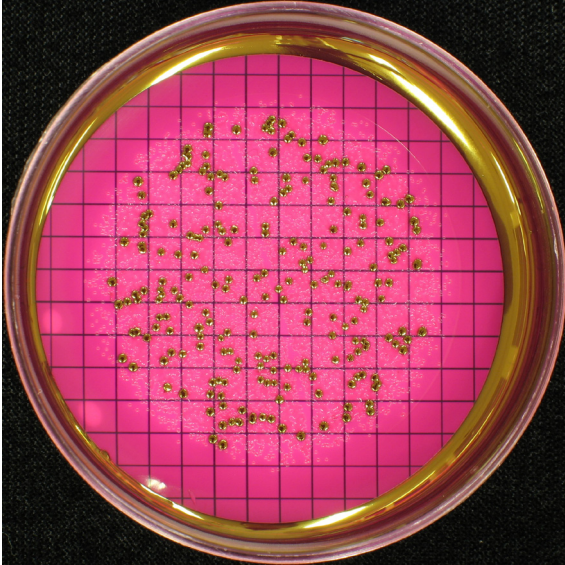


10 ml, 7 days



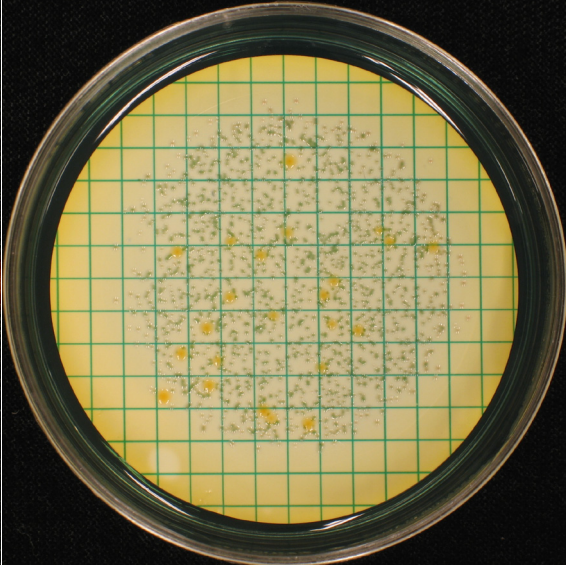
Mixture B

m-Endo Agar LES, 37 °C



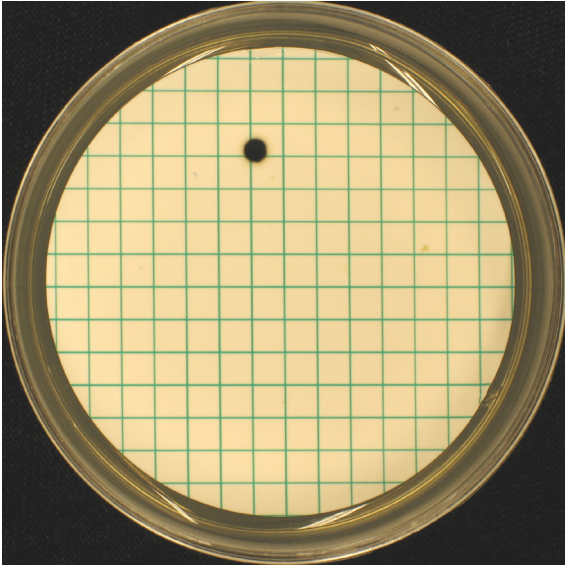
100 ml

m-Lactose TTC Agar, 37 °C



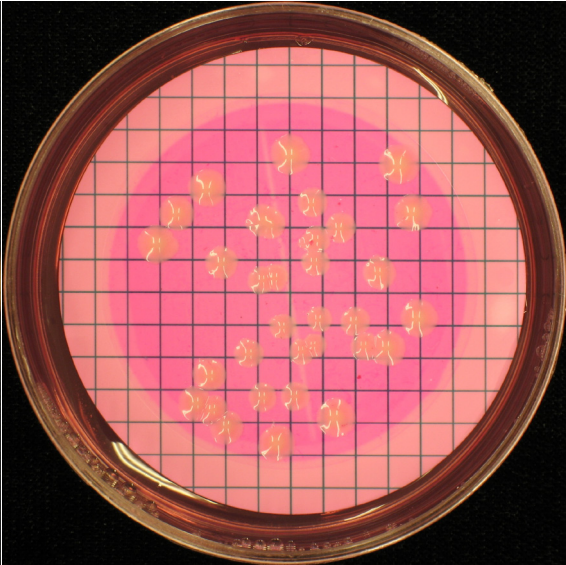
10 ml

m-FC Agar, 44 °C



100 ml

m-Lactose TTC Agar, 44 °C



100 ml, 7 days

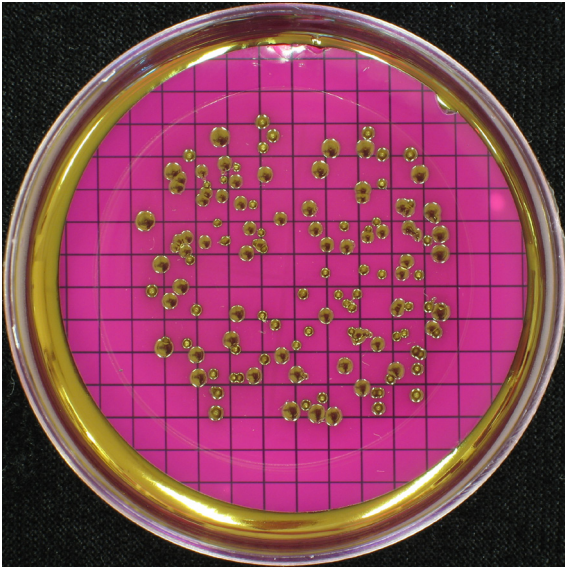
m-TSC Agar, 44 °C

m-Burman Agar, 25 °C



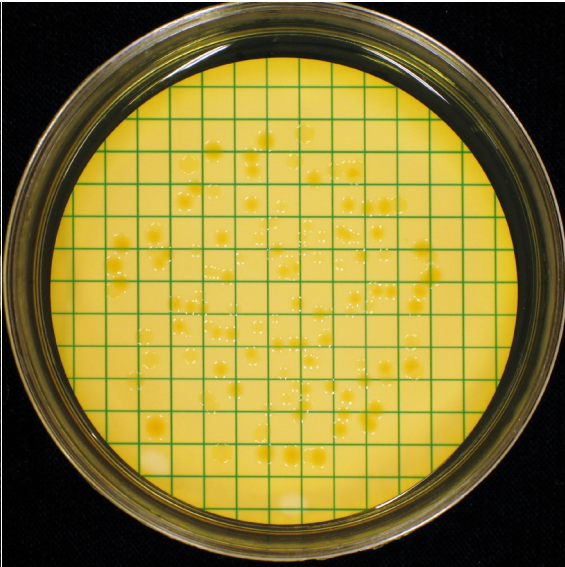
Mixture C

m-Endo Agar LES, 37 °C



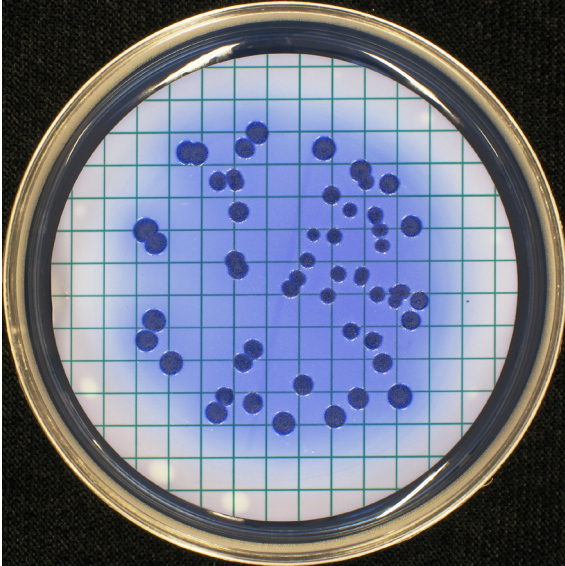
10 ml

m-Lactose TTC Agar, 37 °C



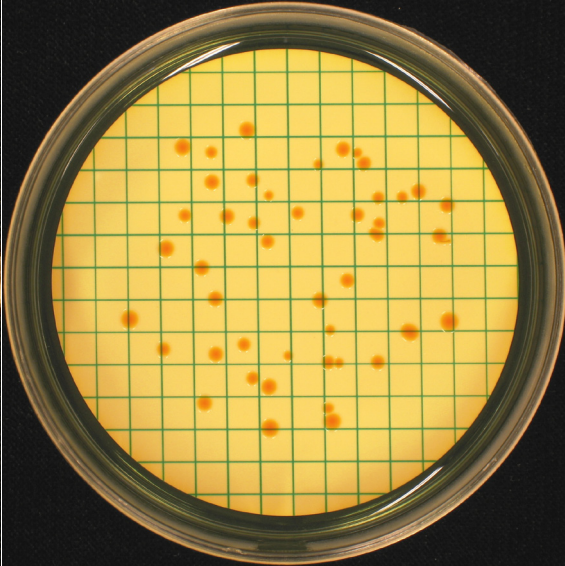
10 ml

m-FC Agar, 44 °C



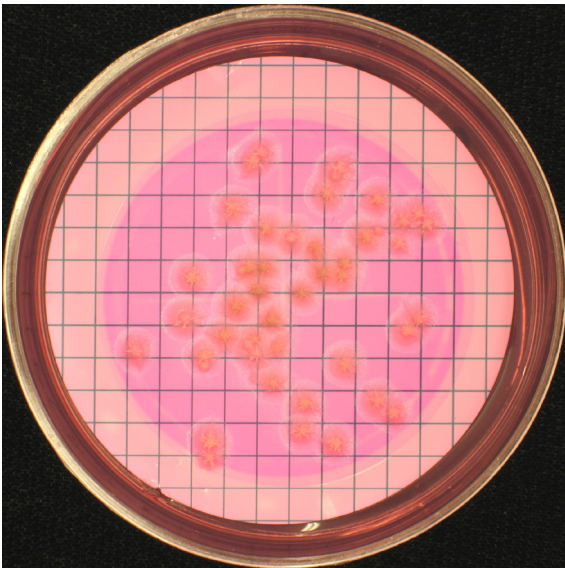
10 ml

m-Lactose TTC Agar, 44 °C



10 ml

m-TSC Agar, 44 °C



100 ml, 7 dygn

m-Burman Agar, 25 °C





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 <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		
													-1.100	0.827	1124	
-0.126	-0.041	0.000						*				0.646	0.143	0.365	1131	
												0.646	-0.668	-3.144	1132	
												2.361	-0.582		1149	
												-4.000	-4.000	-4.000	1237	
						1.654	0.000	-0.004	-0.286	-0.252	0.000	0.103	0.458	0.646	1254	
			0.934									0.103	-3.275	0.915	1290	
0.553	1.637	0.000	0.012	0.688	0.000	0.068	0.000	0.531	1.461	0.859	0.000	0.646	0.143	-0.699	1545	
												-0.805	0.000	-0.356	1594	
								0.000	0.369	-0.617	0.624	0.000	0.903	-0.292	0.267	1611
-0.897	-0.462	0.000				-0.344	0.000	0.192	0.675	-0.252	0.000	-1.498	-0.823	0.068	1753	
0.488	1.852	0.000				0.872	0.000	-0.226	-0.483	1.745	0.000	-0.185	-0.145	-0.467	1868	
-1.811	-0.462	0.000	-1.269	-0.257	0.000	-0.481	0.000	-1.659	0.470	-1.234	0.000	-1.882	0.107	-0.246	1970	
0.357	-1.010	0.000				-0.085	0.000	-0.004	0.835	0.974	0.000	0.380	-0.218	2.185	2050	
-2.476	-2.334	0.000	-1.822	-1.884	0.000							-1.141	-0.181	1.419	2386	
												4.000	4.000		2670	
			0.251	1.160	0.000							-1.141	-0.403	-1.071	2704	
												0.903	0.963	-0.699	2745	
0.016	-0.041	0.000	0.251	0.109	0.000	-3.183	0.000	0.824	-4.000	-0.658	0.000	3.108	1.383	0.460	3042	
															3055	
								0.000	-1.659	-1.029	4.000	0.000	0.103	-1.552	-0.356	3076
			-1.822	-0.734	0.000								0.646	-1.020	1.002	3159
-0.272	0.626	0.000				-1.001	0.000	0.369	0.452	-1.386	0.000	0.646	-0.366	0.554	3162	
-0.815	-1.010	0.000				-1.721		-1.659	-1.242	-1.861	0.000	0.380	0.458	1.087	3305	
4.000	-0.041		1.679	0.109	0.000							0.903	1.447	1.002	3339	
			-1.024	0.417	0.000							0.646	0.178	0.737	3475	
												1.152	0.354	-1.626	3511	
												4.000	-3.705	1.338	3533	
												-0.185	-0.403	0.737	3588	
												0.103	0.560	0.915	3730	
-0.272	-0.041	0.000	0.012	0.109	0.000	0.214	0.000	0.531	0.888	0.624	0.000	0.646	0.458	-0.035	3868	
1.677	-0.041	0.000				0.710	0.000	-0.004	0.205	1.639	0.000	2.709	-1.180	-0.582	4015	
												0.103	-2.299	-0.467	4064	
													-1.261	-0.699	4180	
												-0.805	-0.516	-1.071	4278	
												0.103	-1.100	-1.071	4288	
												-0.805	-0.366	0.169	4319	
			0.752	0.934	0.000	0.490	0.000	0.531	0.591	-0.658	0.000	0.380	-0.145	0.737	4339	
-0.981	-1.010	0.000				-0.720	0.000	0.192	0.829	0.259	0.000	0.646	0.214	-0.467	4343	
0.016	-2.334	0.000	0.251	-1.884	0.000							0.103	-0.630	0.737	4356	
			-2.033	1.160	0.000							-0.487	0.072	-0.699	4459	
												0.903	0.423	0.267	4539	
												0.103	-0.072	0.554	4633	
												-0.185	-0.630	0.267	4713	
0.488	-0.041	0.000				0.992	0.000	0.959	-0.092	1.312	0.000	-0.487	1.159	-0.035	4723	
	4.000	0.000				-0.395	0.000	0.531	-1.029	-0.941	0.000	-1.141	0.797	-1.203	4889	
			0.480	0.688	0.000							0.103	0.249	-0.139	4980	
0.928	0.314	0.000	0.698	0.417	0.000	0.490	0.000	0.531	-2.633	-0.121	0.000	0.903	0.930	0.365	5018	
						0.621	0.000	-1.659		4.000		-0.487	0.036	-2.107	5094	
0.617	-0.462	0.000	0.752	-0.257	0.000	0.872	0.000	0.824	-1.314	-0.252	0.000	-0.185	0.458	0.554	5120	
												-0.805	-0.255	-1.939	5188	
												-0.185	-0.036	-1.480	5201	
												0.903	-1.100	-1.626	5220	
1.283	1.637	0.000	1.306	1.567	0.000	-0.778	0.000	0.192	-0.842	0.383	0.000	-1.498	-0.292	-0.356	5447	
												1.627	0.388	1.256	5553	
0.680	-0.462	0.000				-0.571	0.000	-0.004	0.347	0.259	0.000	-1.141	-0.403	-0.467	5701	
-0.199	-0.041	0.000	0.073	0.109	0.000	0.068	0.000	0.369	-1.156	-1.541	0.000	1.393	1.320	1.419	5950	
0.617	0.909	0.000	0.752	0.934	0.000							-0.805	0.319	0.827	6180	
												-0.487	0.107	0.554	6253	
												0.380	0.930	1.338	6456	
												-1.882	0.963	-1.203	6731	
			0.960	-1.884								-0.487	0.492	-0.467	7096	
															7191	
															7235	
-0.734	0.314	0.000				-0.148	0.000	0.531	0.286	0.007	0.000	0.646	1.028	1.736	7248	
0.086	1.410	0.000				-0.244	0.000	0.824	0.531	-0.385	0.000	-1.141	1.159	-0.356	7302	
						0.214	0.000	-1.659	-1.100	4.000	0.000	-1.498	0.458	-0.246	7395	
0.488	1.637	0.000	0.645	1.567	0.000							0.380	0.963	0.365	7428	
												-0.185	-0.554	0.068	7442	
												1.152	1.126	-0.139	7497	
0.016	-0.462	0.000	0.251	-0.257	0.000							0.103	-0.668	0.554	7596	
0.290	-0.041	0.000	0.480	0.109	0.000	1.654	0.000	4.000	0.029	-1.699	0.000	-0.487	1.191	0.365	7626	
			1.111	-0.734	0.000							1.152	0.897	0.915	7688	
												0.380	0.354	-0.035	7728	
-0.126	0.314	0.000				0.872	0.000	2.043	0.947	-0.385	0.000	-1.882	0.388	-1.071	7876	
0.155	-1.010	0.000	0.367	-0.734	0.000	0.354	0.000	-1.659	0.036	0.974	0.000	-0.487	-1.939	-0.820	7896	
-0.199	-0.041	0.000	0.073	0.109	0.000							-0.185	1.094	-0.582	7930	
												-0.617	0.259	0.000	7962	
-0.272	-0.462	0.000	0.012	-0.257	0.000	1.389	0.000	1.326	2.008	0.859	0.000	0.903	-0.516	-0.582	7968	
-1.613	-2.334	0.000	-1.104	-1.884	0.000							-0.185	2.935	-0.467	8068	
												-1.141	-1.426	0.827	8255	
3.080	1.168		0.857	0.688	0.000	-1.721	0.000	-1.659	-0.351	0.134	0.000	0.380	-0.403	0.646	8260	

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
8329	1 3 2				0.902	0.328	0.059				0.687	0.000	-0.377						
8365	2 3 1				-1.377	-3.287	-4.000						-4.000	-3.910	-2.986	-4.000			-4.000
8380	1 2 3				-0.240	-0.286	3.645				0.283	0.000	2.193	-1.588	-1.516	-1.347	-0.393	0.000	-0.708
8435	2 1 3				-1.065	-0.286	-0.419				-0.376	0.000	0.423						
8569	1 2 3				-0.325	-0.099	1.548				-0.663	0.000	-0.868						
8598	3 1 2																		
8626	2 3 1				1.173	-0.099	1.086				1.256	0.000	1.225						
8628	2 3 1				0.396	-0.099	-0.419				0.189		0.423						
8663	3 1 2				0.087	1.070	1.086				-0.321	0.000	0.215	0.523	1.223	-0.153	-0.557	0.000	-1.842
8742	2 1 3																		
8751	3 2 1																		
8766	1 3 2				0.508	-0.007	-0.180				0.330	0.000	0.702	-0.431	-0.770	-0.498	-0.726	0.000	1.176
8862	3 1 2				0.508	0.900	0.689				0.687	0.000	-0.335	0.331	1.299	0.693	1.025	0.000	0.194
8898	3 2 1				1.337	0.678	1.412				1.628	0.000	0.443	0.757	0.355	0.155	0.070	0.000	-0.584
8955	2 3 1													-0.066	0.203	-1.267	-0.234	0.000	-0.979
9002	1 2 3				-2.577	0.259					-0.604	0.000	1.874						
9359	3 2 1				0.282	-0.481	-0.636				0.236	0.000	-0.136	-0.538	-0.252	1.148	-1.085	0.000	1.456
9436	3 1 2				0.545	-1.567	-0.419				0.189	0.000	-2.242	0.380	-2.820	-1.087	0.498	0.000	-1.006
9451	2 3 1				-0.325	0.430	-0.858				0.467	0.000	-0.661						
9465	2 1 3				0.205	0.083	0.156				-0.008	0.000	-0.416	-0.015	2.088	0.236	-1.085	0.000	-0.726
9569	1 3 2				0.690	0.119	0.606				0.980	0.000	0.172	0.086	0.440	0.690	0.359	0.000	-0.453
9655	1 3 2													-2.773	-0.744	-0.879	1.858		-1.258
9736	2 1 3				0.166	-0.173	1.255				0.330	0.000	-0.150	-0.871	-0.324	-0.823	-1.905	0.000	-0.979
9897	2 1 3				0.434	0.613	1.086				0.189	0.000	0.818	-0.647	-0.300	-2.027	-0.393	0.000	-0.273
9899	3 1 2				0.545	0.224	0.882				0.557	0.000	0.151						
9903	2 3 1				1.239	0.276	0.512				-0.008	0.000	-0.682						
n					89	87	88	0	0	0	85	83	85	62	62	62	62	61	63
Min					-4.000	-3.287	-4.000				-3.486	0.000	-4.000	-4.000	-4.000	-4.000	-4.000	0.000	-4.000
Max					4.000	4.000	4.000				4.000	0.000	2.648	4.000	2.088	3.149	4.000	0.000	4.000
Median					0.205	0.119	0.082				0.091	0.000	-0.042	0.035	0.081	-0.153	-0.080	0.000	-0.162
Mean					0.045	0.046	-0.046				0.006	0.000	-0.094	0.001	-0.065	-0.129	-0.054	0.000	-0.063
SD					1.229	1.083	1.579				1.145	0.000	1.161	1.404	1.114	1.214	1.286	0.000	1.312
z<-3					2	2	6				2	0	3	2	1	2	2	0	2
-3≤z<-2					4	3	2				4	0	2	3	2	2	0	0	0
-2<z≤3					1	0	0				2	0	2	1	1	2	2	0	2
z>3					2	2	4				1	0	0	2	0	1	1	0	1

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	
0.488	0.909	0.000				-0.023	0.000	-0.226	0.976	-0.121	0.000	-0.185	1.415	0.915	8329
						<b>4.000</b>		-1.659	<b>-2.467</b>	0.259		<b>4.000</b>	<b>-2.919</b>	-1.939	8365
															8380
0.988	-0.041	0.000	1.061	0.109	0.000							-0.185	0.830	-0.467	8435
												0.903	0.797	1.812	8569
												-1.141	-0.516	0.169	8598
												1.393	-0.516	-1.779	8626
			-1.822	-1.884	0.000	0.068	0.000	-1.659	0.286	-0.520		0.103	0.696	<b>-3.421</b>	8628
												-1.141	-0.255	1.087	8663
												-0.487	1.126	1.500	8742
												-1.882	0.143	-0.582	8751
0.357	0.314	0.000				-0.378	0.000	1.438	-0.286	-0.520	0.000	-0.185	-0.516	-0.582	8766
0.553	-0.041	0.000				-0.682	0.000	0.192	0.561	0.859	0.000	0.903	-0.940	0.915	8862
0.680	-0.041	0.000				0.932	0.000	1.086	1.847	0.624	0.000	-0.185	1.320	0.646	8898
			0.424	0.109	0.000	0.354	0.000	-0.004	1.461	1.201	0.000	0.903	0.797	-1.071	8955
												-0.185	-0.440	0.365	9002
1.048	1.168	0.000		1.160	0.000	1.334	0.000	0.824	-0.028	1.312	0.000	1.627	-0.630	-1.071	9359
-1.914	0.909	0.000	-1.443	0.934	0.000	<b>-2.245</b>	0.000	-1.659	-1.156	<b>-3.134</b>	0.000	-1.882	-1.343	0.460	9436
-1.331	-1.010	0.000	-0.795	-0.734	0.000							1.627	0.763	0.068	9451
												0.903	<b>4.000</b>	1.888	9465
-0.734	-1.010	0.000	-0.372	-0.734	0.000	0.354	0.000	0.824	0.409	0.383	0.000	-1.882	0.595	-0.582	9569
												<b>-4.000</b>	-1.426	-1.939	9655
0.290	-0.041	0.000				0.872	0.000	0.192	-0.279	0.624	0.000	-0.487	0.036	1.002	9736
												0.646	-0.440	0.267	9897
-0.897	0.626	0.000				0.023	0.000	0.192	0.752	0.007	0.000	0.903	0.458	0.554	9899
-0.575	0.626	0.000				-0.535	0.000	0.192	0.092	0.259	0.000	-1.498	-0.668	-1.480	9903
45	46	44	34	36	34	43	44	46	45	46	43	100	104	104	n
-2.476	-2.334	0.000	-2.033	-1.884	0.000	-3.183	0.000	-1.659	-4.000	-3.134	0.000	-4.000	-4.000	-4.000	Min
4.000	4.000	0.000	1.679	1.567	0.000	4.000	0.000	4.000	2.008	4.000	0.000	4.000	4.000	4.000	Max
0.086	-0.041	0.000	0.251	0.109	0.000	0.068	0.000	0.192	0.036	0.259	0.000	0.103	0.054	0.068	Median
0.089	0.087	0.000	0.000	0.000	0.000	0.093	0.000	0.087	-0.089	0.261	0.000	0.000	0.003	-0.033	Mean
1.154	1.151	0.000	1.000	1.000	0.000	1.161	0.000	1.151	1.154	1.389	0.000	1.267	1.249	1.181	SD
0	0	0	0	0	0	1	0	0	1	1	0	2	3	3	Summa
1	3	0	1	0	0	1	0	0	2	0	0	0	2	1	35
0	0	0	0	0	0	0	0	1	1	0	0	1	2	1	33
2	1	0	0	0	0	1	0	1	0	3	0	3	2	1	19
															28



1. Proficiency Testing – Food Chemistry, Lead and cadmium extracted from ceramics by C Åstrand and Lars Jorhem.
2. Fullkorn, bönor och ägg – analys av näringsämnen av C Gard, I Mattisson, A Staffas och C Åstrand.
3. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N 45 by L Merino.
4. Proficiency Testing – Food Microbiology, January 2010 by C Normark and K Mykkänen.
5. Riksprojekt 2009. Salmonella, Campylobacter och E.coli i färska kryddor och bladgrönsaker från Sydostasien av N Karnehed och M Lindblad.
6. Vad gör de som drabbas av magsjuka och matförgiftningar – resultat från en nationell intervjuundersökning av J Toljander och N Karnehed.
7. The Swedish Monitoring of Pesticide Residues in Food of Plant Origin: 2008, Part 1 – National Report by A Andersson, F Broman, A Hellström and B-G Österdahl.  
The Swedish Monitoring of Pesticide Residues in Food of Plant Origin: 2008, Part 2 – Report to Commission and EFSA by A Andersson and A Hellström.
8. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T-20 by C Åstrand and Lars Jorhem.
9. Proficiency Testing – Drinking Water Microbiology, 2010:1, March by C Lantz, T Šlapokas and M Olsson.
10. Rapportering av livsmedelskontrollen 2009 av D Rosling och K Bäcklund Stålenheim.
11. Rapportering av dricksvattenkontrollen 2009 av D Rosling.
12. Proficiency Testing – Food Microbiology, April 2010 by C Normark, K Mykkänen and I Boriak.
13. Kontroll av rests substanser i levande djur och animaliska livsmedel. Resultat 2009 av I Nordlander, B Aspenström-Fagerlund, A Glynn, A Johansson, K Granelli, E Fredberg, I Nilsson, Livsmedelsverket och K Girma, Jordbruksverket.
14. Metaller i fisk i Sverige – sammanställning av analysdata 2001-2005 av B Sundström och L Jorhem.
15. Import av fisk från tredje land – redlighetsprojekt inom gränskontrollen av E Fredberg, P Elvingsson och Y Sjögren.
16. Djurskydd vid slakt – ett kontrollprojekt av C Berg och T Axelsson.
17. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N 46 by L Merino.
18. Proficiency Testing – Food Chemistry, Vitamins in Food, Round V-8 by H S Strandler and A Staffas.
19. Potatis – analys av näringsämnen av V Öhrvik, I Mattisson, S Wretling och C Åstrand.
20. Proficiency Testing – Drinking Water Microbiology, 2010:2, September by C Lantz, T Šlapokas and I Boriak.
21. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T-21 by C Åstrand and Lars Jorhem.
22. Rapport från GMO-projektet 2010. Undersökning av förekomsten av icke godkända GMO i livsmedel av Z Kurowska.
23. Proficiency Testing – Food Microbiology, October 2010 by C Normark, K Mykkänen and I Boriak.

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 kunskapssammanställning 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 Lars Jorhem.
9. Riksprojekt 2010. *Listeria monocytogenes* i kyld ätferdig 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. Proficiency Testing – Drinking Water Microbiology, 2011:1, March by T Šlapokas, C Lantz and M Lindqvist.

