

# Proficiency testing Drinking water Microbiology

September 2022

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*Recommended citation*

Blom, L. 2022. PT Drinking water Microbiology – September 2022, Swedish Food Agency, Uppsala, Sweden.

*Edition*

Version 1 (2022-11-16)

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PT Drinking water September 2022 is registered as no. 2022/03191 at the Swedish Food Agency



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# Abbreviations

## Media

BEAA	Bile Esculin Azide Agar (EN ISO 7899-2:2000)
CCA	Chromogenic Coliform Agar (EN ISO 9308-1:2014)
Colilert	Colilert® Quanti-Tray® (IDEXX Inc.; EN ISO 9308-2:2014)
Enterolert	Enterolert® Quanti-Tray® (IDEXX Inc.)
LES	m-Endo Agar LES (SS 028167)
LTLNB	Lactose tryptone lauryl sulphate broth (SS 028167)
m-Ent	m-Enterococcus Agar (EN ISO 7899-2:2000)
m-FC	m-FC Agar (SS 028167)
PACN	Pseudomonas Agar base/CN agar (EN ISO 16266:2008)
PCA	Plate count agar
Pseudalert	Pseudalert® Quanti-Tray® (IDEXX Inc.; ISO 16266-2:2018)
YEA	Yeast extract Agar (EN ISO 6222:1999)

## Other abbreviations

MF	Membrane filter (method)
MPN	Most Probable Number (quantification based on statistical distributions)
ISO	International Organization for Standardization
EN	European standard from "Comité Européen de Normalisation" (CEN)
DS, NS, SFS, SS	National standards from Denmark, Norway, Finland and Sweden
SLV	Livsmedelsverket/Swedish Food Agency, Sweden

# Analyses in this PT round

## Quantitative analyses

Coliform bacteria

*Escherichia coli*

Suspected thermotolerant coliform bacteria (not assessed)

Intestinal enterococci

*Pseudomonas aeruginosa*

Culturable microorganisms, 72 hours incubation at  $22 \pm 2$  °C

Culturable microorganisms, 48 hours incubation at  $36 \pm 2$  °C

# Method

## Reporting of results and method information

It is the responsibility of the individual participants to correctly report results according to the instructions. It is also mandatory for the participants to report method information for all analyses. This method information is sometimes contradictory or difficult to interpret. For example when participants state a medium that is not included in the standard method they refer to, or when manual comments by the participant contradict the reported method information. In such cases, the reported method information provided by the participants is generally used in method comparisons “as it is”. Alternatively, method data that are difficult to interpret may be excluded or added to the group “Other”, together with results from methods and media that are only used by 1–2 participants.

## Standard deviation and assigned value

Results reported by participants as “> value” are not evaluated. Results reported as “< value” are treated as zero (negative result). Unless otherwise stated, evaluation of the participants’ results and statistical calculations are carried out on square root transformed results. In the report, re-transformed statistical measures (i.e. in the normal cfu scale) are shown as a practical assistance to the participants.

A robust statistical approach is used to determine the mean value and standard deviation. Algorithm A with iterated scale as described in ISO 13528:2015 [1] is used to determine the robust mean ( $m_{PT}$ ) and robust standard deviation ( $s_{PT}$ ) of the participants’ results. Results that are obviously erroneous are excluded prior to determining  $m_{PT}$  and  $s_{PT}$  (blunder removal). For evaluated parameters, the assigned value consists of  $m_{PT}$ . It is regarded as the true, normative value. For parameters that are not statistically evaluated, the median (Med) of the participants results is instead used as the assigned value. This is normally the case for parameters with fewer than 20 reported results.

## Outliers

Outliers are results that deviate from the other results in a way that cannot be explained by normal variation. Results within  $m_{PT} \pm 3s_{PT}$  are considered acceptable, whereas results outside this interval are considered as outliers. When fewer than 20 participants have reported results, as well as in some individual cases, subjective adjustments are made to set acceptance limits based on prior knowledge of the samples contents.

## Results from different methods

*Non-robust* mean values (m), standard deviations (s) median values (Med) and coefficients of variation (CV) are calculated to assist in the evaluation of the results from different methods. These are shown in tables in the report, in connection with the respective analyses. In these instances, m, s, Med and CV are calculated from the respective method groups’ results, with outliers and false results excluded. Normally, for method groups with fewer than 5 results, only the number of false results and outliers are provided.

## Coefficient of variation

The coefficient of variation (CV) is a relative measure and is calculated as:

$$CV = 100 \times \frac{S_{PT}}{m_{PT}}$$

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

## Measurement uncertainty for the assigned value

The standard uncertainty ( $u_{PT}$ ) of the assigned value ( $m_{PT}$ ) is estimated from the standard deviation ( $s_{PT}$ ) and the number of evaluated results ( $n$ ):

$$u_{PT} = 1,25 \times \frac{S_{PT}}{\sqrt{n}}$$

The measurement uncertainty is considered negligible compared to the standard deviation (which is used for evaluating the participants' results) when:

$$u_{PT} < 0,3s_{PT}$$

When this criterion is not met, assessment of the results should be taken with consideration, since participants might inaccurately receive action and warning signals.

In annex 1 the relative standard uncertainty ( $u_{rel}$ ) of  $m_{PT}$  is also provided.

$$u_{rel,mPT}(\%) = 100 \times \frac{S_{PT}}{\sqrt{n} \cdot m_{PT}}$$

## Z-scores

To allow comparison of the results from different analyses and samples, results are transformed into standard values (z-scores). Z-scores are calculated as:

$$Z = \frac{x_{lab} - m_{PT}}{S_{PT}}$$

where  $x_{lab}$  is the result of the individual participant.

Z-scores for individual analyses are shown in Annex 2 and can be used as a tool by participants when following up on the results. For quantitative analyses, a z-score is either positive or negative, depending on whether the participants result is higher or lower than  $m_{PT}$ .

In evaluations of the analytical results, the following guidelines can be used:

- $|z| \leq 2$  indicates that the result is acceptable
- $2 < |z| < 3$  indicates a warning that the result may be deviating, and might motivate an action in the follow-up process
- $|z| \geq 3$  indicates that the result is regarded as deviating and should lead to an action in the follow-up process

## Table legends

- N number of participants that reported results for the analysis
- n number of participants with acceptable result (false results and outliers excluded)
- m mean value in cfu 100 ml<sup>-1</sup>, cfu ml<sup>-1</sup> for culturable microorganisms
- Med median in cfu 100 ml<sup>-1</sup>
- CV coefficient of variation in percent
- F number of false positive or false negative results
- < number of low outliers
- > number of high outliers

## Figure legends

- results within the interval of acceptance
- outlier
- false negative result
- \* value outside the x-axis scale



# Results

## General outcome

Samples were sent to 81 participants; 34 in Sweden, 45 in European countries, and two outside of Europe. Of the 79 participants that reported results, 34 (43 %) provided at least one result that received an annotation.

Individual results are listed in Annex 1 and on the website: <https://www2.slv.se/absint>. Z-scores for individual results are listed in Annex 2.

**Table 1.** Composition of the test material and proportion of deviating results (N: number of reported results, F: false positive or false negative, X: outliers)

	Sample A				Sample B				Sample C			
<b>% participants with</b>												
<b>Microorganisms</b>	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Lactobacillus plantarum</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus saprophyticus</i>				<i>Klebsiella pneumoniae</i> <i>Enterobacter aerogenes</i> <i>Enterococcus hirae</i> <i>Burkholderia cepacia</i> <i>Staphylococcus capitis</i>				<i>Escherichia coli</i> <i>Aeromonas hydrophila</i> <i>Pseudomonas aeruginosa</i> <i>Pseudomonas fluorescens</i>			
<b>Analysis</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>	<b>Target organism</b>	<b>N</b>	<b>F</b>	<b>X</b>
Coliform bacteria (MF)	<i>E. coli</i> <i>K. pneumoniae</i>	57	1	3	<i>K. pneumoniae</i> <i>E. aerogenes</i>	57	1	2	<i>E. coli</i> ( <i>A. hydrophila</i> )	56	1	1
Coliform bacteria (MPN)	<i>E. coli</i> <i>K. pneumoniae</i>	50	0	4	<i>K. pneumoniae</i> <i>E. aerogenes</i>	50	0	2	<i>E. coli</i>	49	0	2
Susp. thermotolerant coliform bact.(MF)	<i>E. coli</i> <i>K. pneumoniae</i>	22	-	-	<i>K. pneumoniae</i>	22	-	-	<i>E. coli</i>	22	-	-
<i>E. coli</i> (MF)	<i>E. coli</i> ( <i>K. pneumoniae</i> )	57	1	1	( <i>K. pneumoniae</i> )	56	1	0	<i>E. coli</i>	34	0	1
<i>E. coli</i> (MPN)	<i>E. coli</i>	51	1	4	-	51	0	-	<i>E. coli</i> *	48	0	0
Intestinal enterococci	( <i>L. plantarum</i> ) ( <i>S. saprophyticus.</i> )	60	8	0	<i>E. hirae</i>	60	1	3	-	60	1	-
<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	47	1	1	( <i>B. cepacia</i> )	46	0	-	<i>P. aeruginosa</i>	46	3	1
Culturable micro-organisms 22 °C	<i>L. plantarum</i> <i>S. saprophyticus</i>	77	0	5	<i>E. hirae</i> <i>K. pneumoniae</i> <i>E. aerogenes</i>	77	1	4	<i>P. fluorescens</i>	77	0	1
Culturable micro-organisms 37 °C	<i>L. plantarum</i> <i>S. saprophyticus</i>	60	0	0	<i>S. capitis</i> <i>E. hirae</i> <i>K. pneumoniae</i> <i>E. aerogenes</i>	60	1	3	All except <i>P. fluorescens</i>	60	0	2

- no target organism or no value; **microorganism** = main target organism; microorganism = few colonies; (*microorganism*) = false positive before confirmation; \* The strain has a low  $\beta$ -glucuronidase activity and will not be detected as *E. coli* with Colilert.

The results are not evaluated

# Coliform bacteria

## Sample A

The strains of *E. coli* and *K. pneumoniae* were target organisms. Both strains form typical colonies with a metallic sheen on m-Endo Agar LES (LES). On Chromocult Coliform Agar (CCA), *E. coli* and *K pneumoniae* form blue and pink colonies, respectively.

In total, 57 participants reported results for MF methods. One false negative result and three high outliers were reported.

For most probable number (MPN) methods, 50 participants reported results. One high and three low outliers were reported.

## Sample B

The strains of *K. pneumoniae* and *E. aerogenes* were target organisms. On LES, *E. aerogenes* may form red colonies without the characteristic metallic sheen.

In total, 57 participants reported results for MF methods. One false negative result and two high outliers were reported.

For MPN methods, 50 participants reported results. Two low outliers were reported.

## Sample C

The strain of *E. coli* was target organism. The oxidase-positive strain of *A. hydrophila* was present as a false-positive organism for the analysis.

In total, 56 participants reported results for MF methods. One false negative result and one high outlier were reported.

For MPN methods, 49 participants reported results. Two low outliers were reported.

## General remarks

For MF, most participants followed either EN ISO 9308-1:2014 using the enzyme-based chromogenic medium CCA, or Nordic national standards using LES based on lactose fermentation.

The group Other/Unknown in the table includes four different media, from both water and food methods, as well as from methods in the medical field.

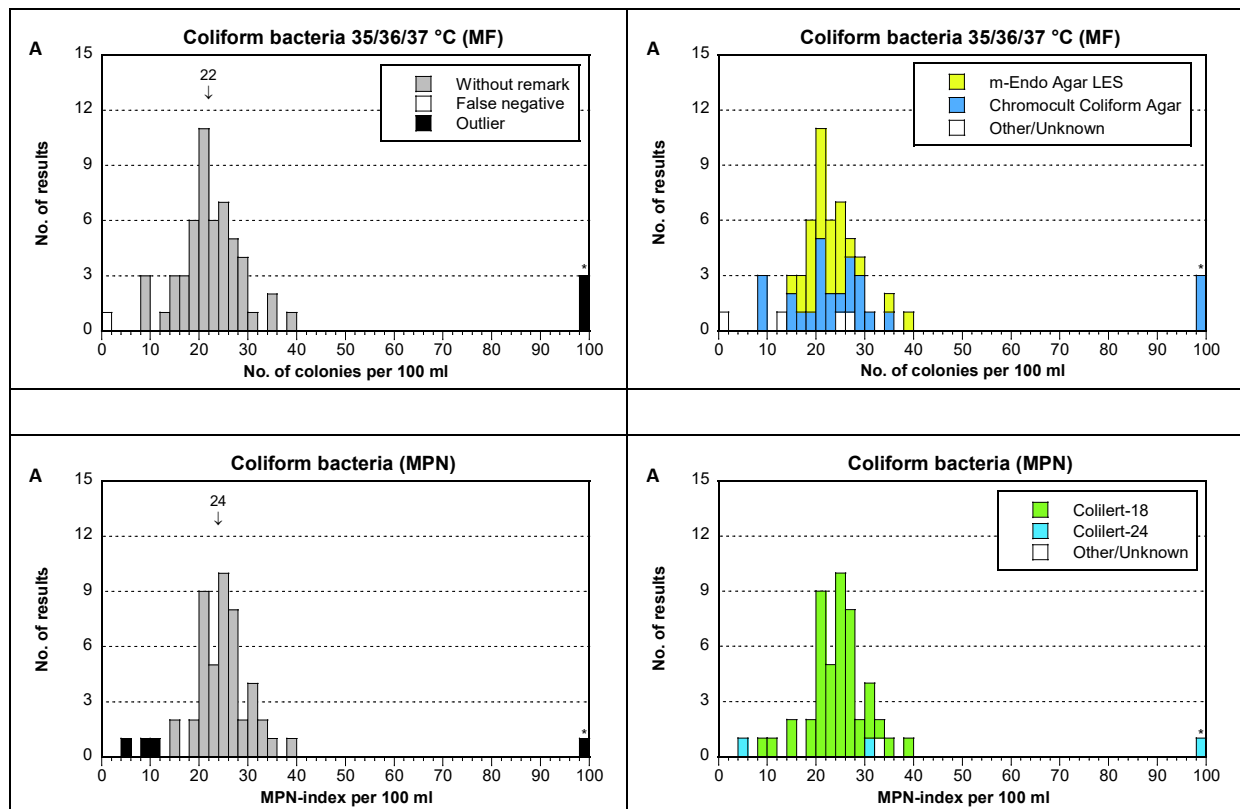
Of the 49 participants that reported Colilert, 94 % used Colilert-18 (ISO 9308-2:2012) for the MPN method. The other 6 % used Colilert-24. Most participants (82 %) used trays with 97 wells. The remaining participants used trays with 51 wells, and one participant used both trays with 51 and with 97 wells. As with CCA, Colilert is based on the activity of a characteristic enzyme found in coliforms,  $\beta$ -D-galactosidase.

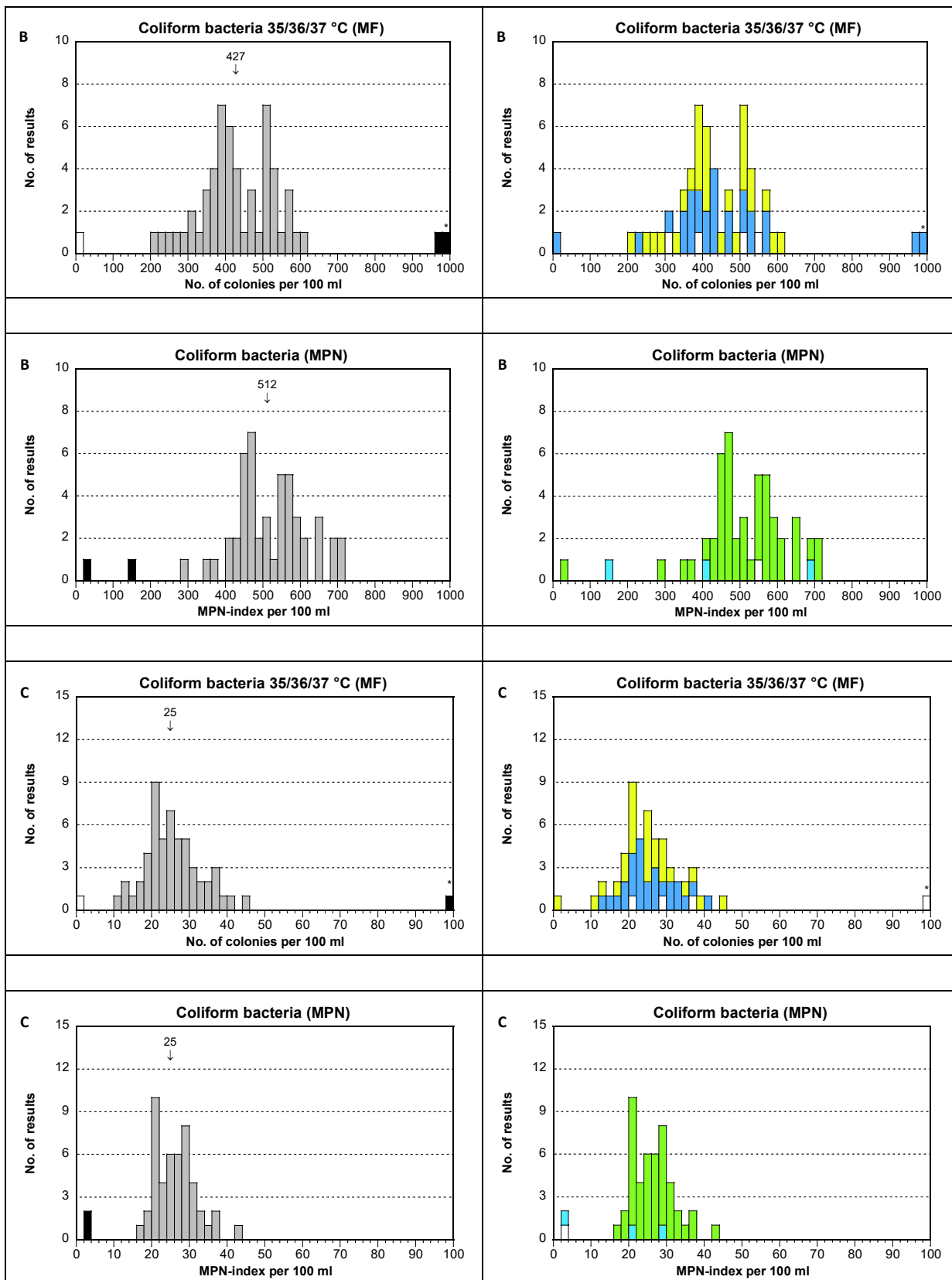
The group Other/Unknown in the table used a multiple tube method based on lactose fermentation for the MPN method.

**Table 2.** Results from analysis of coliform bacteria.

Medium	Sample A						Sample B						Sample C					
	N	n	m	CV	F	< >	N	n	m	CV	F	< >	N	n	m	CV	F	< >
<b>All results, MF</b>	<b>57</b>	<b>53</b>	<b>22</b>	<b>14</b>	<b>1</b>	<b>0 3</b>	<b>57</b>	<b>54</b>	<b>427</b>	<b>12</b>	<b>1</b>	<b>0 2</b>	<b>56</b>	<b>54</b>	<b>25</b>	<b>14</b>	<b>1</b>	<b>0 1</b>
LES	27	27	22	11	0	0 0	27	27	416	13	0	0 0	26	25	24	16	1	0 0
CCA	26	23	21	18	0	0 3	26	23	418	10	1	0 2	26	26	25	14	0	0 0
Other/Unknown	4	3	-	-	1	0 0	4	4	-	-	0 0 0	4	3	-	-	0	0 0 1	
<b>All results, MPN</b>	<b>50</b>	<b>46</b>	<b>24</b>	<b>11</b>	<b>0</b>	<b>3 1</b>	<b>50</b>	<b>48</b>	<b>512</b>	<b>10</b>	<b>0</b>	<b>2 0</b>	<b>49</b>	<b>47</b>	<b>25</b>	<b>11</b>	<b>0</b>	<b>2 0</b>
Colilert-18	46	44	24	10	0	2 0	46	45	516	9	0	1 0	45	45	26	10	0	0 0
Colilert-24	3	1	-	-	0	1 1	3	2	-	-	0 1 0	3	2	-	-	0	1 0	
Other/Unknown	1	1	-	-	0	0 0	1	1	-	-	0 0 0	1	0	-	-	0	1 0	

For "All results", m = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods, m = mean value for the particular method, with outliers and false results excluded.





# *Escherichia coli*

## Sample A

The strain of *E. coli* was target organism. The strain is positive for indole production and  $\beta$ -glucuronidase activity, and it produces gas in LTLSB.

In total, 57 participants reported results for MF methods. One false negative result and one high outlier were reported.

For MPN methods, 51 participants reported results. One false negative result, as well as one low and three high outliers were reported.

## Sample B

No target organism was present in the sample.

In total, 56 participants reported results for MF methods and 51 participants reported results for MPN methods. For MF methods, one false positive result was reported.

## Sample C

The sample contained a strain of *E. coli*. The strain has a low  $\beta$ -glucuronidase activity. It will therefore form atypical pink to violet colonies on CCA, and will not be detected as *E. coli* with Colilert. The strain of *E. coli* produces both gas and indole in LTLSB.

**For participants that followed methods based on  $\beta$ -glucuronidase activity, not detecting *E. coli* is considered as a correct result. These results have therefore been excluded from the statistical evaluation. Similarly, positive results reported by participants that used MPN methods are considered correct, and have been excluded from the statistical evaluation.**

In total, 56 participants reported results for MF methods. Of these, 22 results were excluded. One low outlier was reported. Of the excluded results, 18 participants used CCA and the other four used confirmation based on  $\beta$ -glucuronidase activity.

For MPN methods, 51 participants reported results. Three of these were excluded. These used a different method, included an additional typing or confirmation.

## General remarks

Most participants followed EN ISO 9308-1:2014, Nordic national standards (see table) and/or ISO 9308-2:2012. The definition of *E. coli* differs between the methods. EN ISO 9308-1:2014 defines *E. coli* as bacteria that form typical blue to violet colonies on CCA, with no additional confirmation. ISO 9308-2:2012, defines *E. coli* as yellow wells that also exhibit any degree of fluorescence on Colilert (also  $\beta$ -glucuronidase positive).

When colonies are isolated from LES or m-FC, confirmation is required. Depending on the method, tests for indole production and/or  $\beta$ -glucuronidase activity are usually performed from oxidase-negative

presumptive colonies. In general, confirmation appears to have been performed when required by the method.

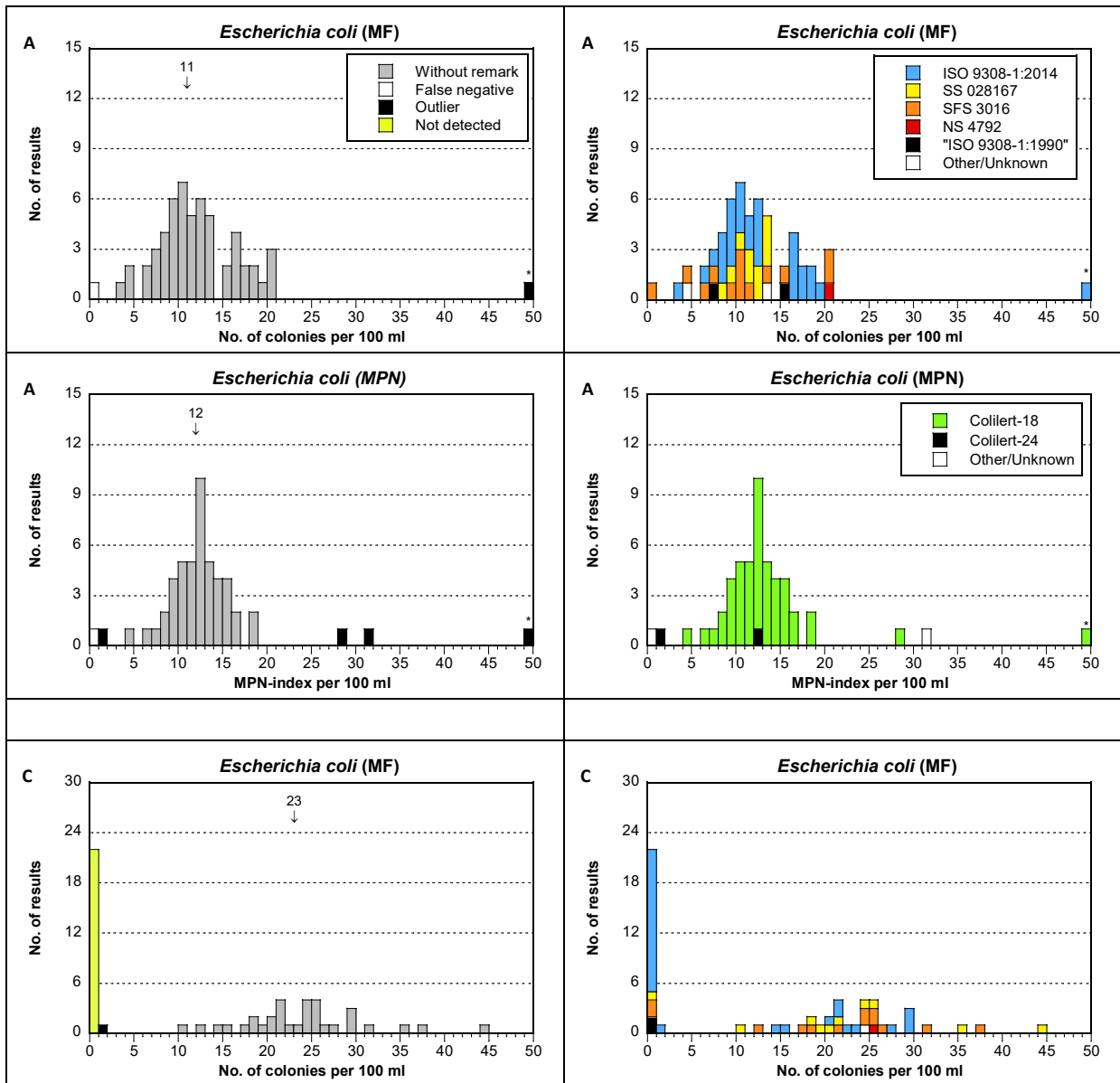
The primary MF growth media CCA and LES are used at 35/36/37 °C and m-FC at 44/44.5 °C.

**Table 3.** Results from analysis of *Escherichia coli*.

	Sample A							Sample B							Sample C							
	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	N	n	m	CV	F <sup>1</sup>	<	>	
<b>All results MF</b>	<b>57</b>	<b>55</b>	<b>11</b>	<b>20</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>56</b>	<b>55</b>	<b>0</b>	-	<b>1</b>	-	-	<b>56</b>	<b>33</b>	<b>23</b>	<b>14</b>	<b>22</b>	<b>1</b>	<b>0</b>	
Colony origin																						
36 ± 2 °C	38	37	11	20	1	0	0	37	37	0	-	0	-	-	37	20	23	18	16	1	0	
44/44.5 °C	8	8	11	23	0	0	0	8	7	0	-	1	-	-	8	6	23	8	2	0	0	
36 ± 2 & 44/44.5 °C	9	8	12	15	0	0	1	9	9	0	-	0	-	-	9	6	22	14	3	0	0	
Other/Unknown	2	2	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0	
Standard																						
ISO 9308-1:2014	29	28	11	19	0	0	1	29	29	0	-	0	-	-	29	11	22	12	17	1	0	
SS 028167	10	10	11	8	0	0	0	10	9	0	-	1	-	-	10	9	23	21	1	0	0	
SFS 3016 (4088)	13	12	11	23	1	0	0	13	13	0	-	0	-	-	13	11	23	15	2	0	0	
NS 4792	1	1	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0	
"ISO 9308-1:1990"	2	2	-	-	0	0	0	2	2	-	-	0	-	-	2	0	-	-	2	0	0	
Other/Unknown	2	2	-	-	0	0	0	1	1	-	-	0	-	-	1	1	-	-	0	0	0	
<b>All results MPN</b>	<b>51</b>	<b>46</b>	<b>12</b>	<b>14</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>51</b>	<b>51</b>	<b>0</b>	-	<b>0</b>	-	-	<b>48</b>	<b>48</b>	<b>0</b>	-	<b>3</b>	-	-	
Colilert-18	47	45	12	13	0	0	2	47	47	0	-	0	-	-	47	46	0	-	1	-	-	
Colilert-24	2	1	-	-	0	1	0	2	2	-	-	0	-	-	2	2	-	-	0	-	-	
Other, MPN	2	0	-	-	1	0	1	2	2	-	-	0	-	-	2	0	-	-	2	-	-	

For "All results", m = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods, m = mean value for the particular method, with outliers and false results excluded.

<sup>1</sup> Correct result according to participants' method.



# Suspected thermotolerant coliform bacteria

## Sample A

The strains of *E. coli* and *K. pneumoniae* were target organisms. Both strains form blue colonies on m-FC Agar at 44/44.5 °C.

In total, 22 participants reported results.

## Sample B

The strain of *K. pneumoniae* was target organism. On m-FC Agar, the strain of *E. aerogenes* may form small translucent colonies with a blue centre.

In total, 22 participants reported results. Two participants did not detect any suspected thermotolerant coliform bacteria.

## Sample C

The strain of *E. coli* was target organism. On m-FC Agar, it forms typical blue colonies at 44/44.5 °C.

In total, 22 participants reported results.

## General remarks

In total, 22 participants reported results. Of these, 18 participants (86 %) incubated on m-FC at 44 or 44.5 °C.

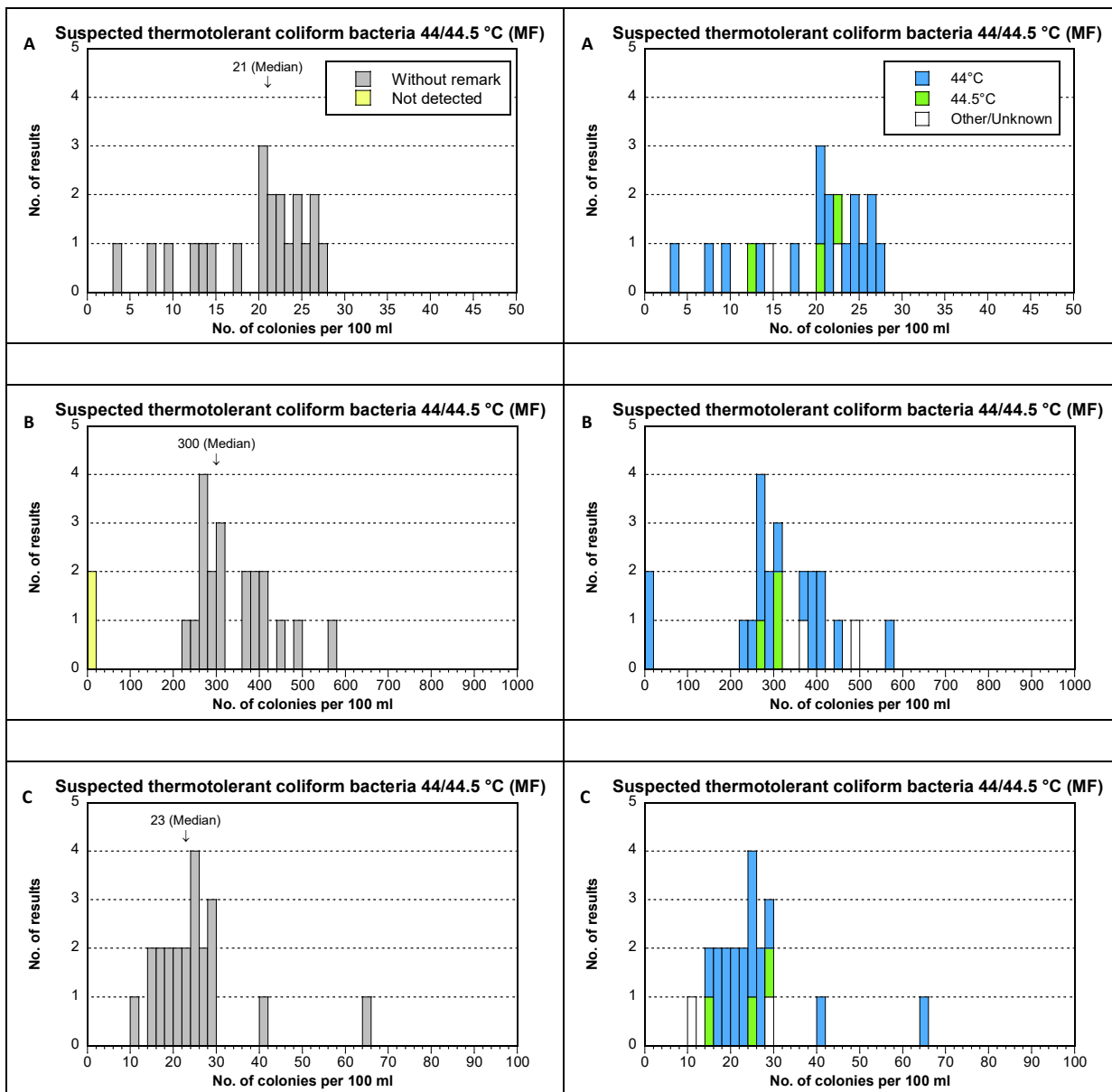
The definition of suspected thermotolerant coliform bacteria is typical colonies on MF at 44/44.5 °C. The two participants in the group Other/Unknown have stated methods where the primary media are incubated at 35/36/37 °C.

The parameter is not statistically evaluated (see page 6). Therefore, no outliers are identified and the median of the participants' results is presented instead of the robust mean value.

**Table 4.** Results from analysis of suspected thermotolerant coliform bacteria.

	Sample A							Sample B							Sample C						
	N	n	Med	CV	F	<	>	N	n	Med	CV	F	<	>	N	n	Med	CV	F	<	>
All results	22	22	21	–	–	–	–	22	22	300	–	–	–	–	22	22	23	–	–	–	–
44 °C	17	17	21	–	–	–	–	17	17	293	–	–	–	–	17	17	22	–	–	–	–
44.5 °C	3	3	–	–	–	–	–	3	3	–	–	–	–	–	3	3	–	–	–	–	–
Other/Unknown	2	2	–	–	–	–	–	2	2	–	–	–	–	–	2	2	–	–	–	–	–





# Intestinal enterococci

## Sample A

No target organism was present in the sample. The strains of *L. plantarum* and *S. saprophyticus* were present as false-positive organisms for the analysis. The strain of *L. plantarum* forms colonies after two days on m-Ent and may show a weak reaction on BEAA.

In total, 60 participants reported results. Eight false positive results were reported.

## Sample B

The strain of *E. hirae* was target organism. On m-Ent, it forms light red colonies. Upon confirmation on BEAA, a distinct black colour is typically seen.

In total, 60 participants reported results. One false negative result, as well as two high and one low outliers were reported.

## Sample C

No target organism was present in the sample.

In total, 60 participants reported results. One false positive result was reported.

## General remarks

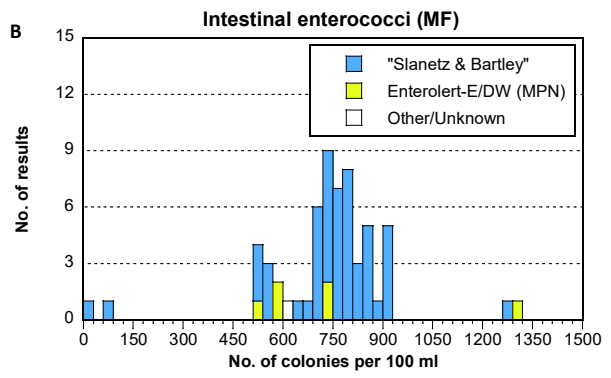
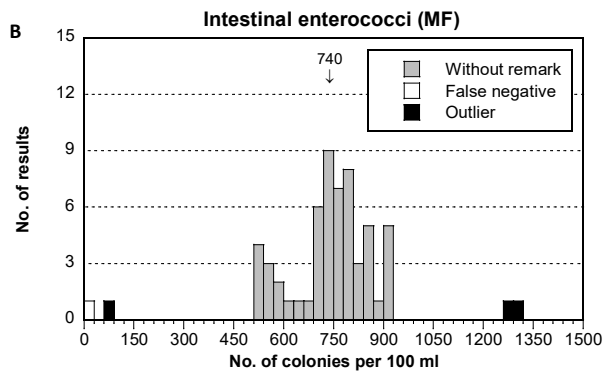
Most participants (88 %) followed EN ISO 7899-2:2000 using m-Ent (Slanetz & Bartley). Six participants used Enterolert® (Idexx Inc.). Three of these used Enterolert®-E (Idexx Inc.) and the other two used Enterolert®-DW (Idexx Inc.). The group Other/Unknown in the table used Enterococcus Selective Agar

The incubation temperature for m-Ent was 35, 36 or 37 °C, except for one participant that incubated at 41 °C. The incubation temperature was 41 °C for Enterolert and 37 °C for the Enterococcus Selective Agar.

**Table 5.** Results from analysis of Intestinal enterococci.

Medium	Sample A							Sample B							Sample C						
	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>
<b>All results</b>	<b>60</b>	<b>52</b>	<b>0</b>	–	<b>8</b>	–	–	<b>60</b>	<b>56</b>	<b>740</b>	<b>8</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>60</b>	<b>59</b>	<b>0</b>	–	<b>1</b>	–	–
Slanetz & Bartley	53	46	0	–	7	–	–	53	50	750	7	1	1	1	53	52	0	–	1	–	–
Enterolert	6	5	0	–	1	–	–	6	5	628	7	0	0	1	6	6	0	–	0	–	–
Other/Unknown	1	1	–	–	0	–	–	1	1	–	–	0	0	0	1	1	–	–	0	–	–

For “All results”, m = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods, m = mean value for the particular method, with outliers and false results excluded.



# *Pseudomonas aeruginosa*

## Sample A

The strain of *P. aeruginosa* was target organism. On PACN, it forms typical blue-green colonies that exhibit fluorescence under UV light.

In total, 47 participants reported results. One false negative result and one high outlier were reported.

## Sample B

No target organism was present in the sample. On PACN, *B. cepacia* may form transparent colonies.

In total, 46 participants reported results.

## Sample C

The strain of *P. aeruginosa* was target organism. On PACN, it forms green colonies with some red-brown pigment. The strain exhibits fluorescence under UV-light.

In total, 46 participants reported results. Three false negative results and one high outlier were reported.

## General remarks

Most participants (74 %) followed EN ISO 16266:2008. Since unhealthy substances like mercury are included, many laboratories have modified the standard and replaced the confirmation tests by another method. However, when only typical blue-green (pyocyanin-producing) colonies are present, no confirmation is required.

Pseudalert® (Idexx Inc.) was used by 10 participants. For sample C, two false negative results were reported for Pseudalert and the mean was lower compared to EN ISO 16266:2008.

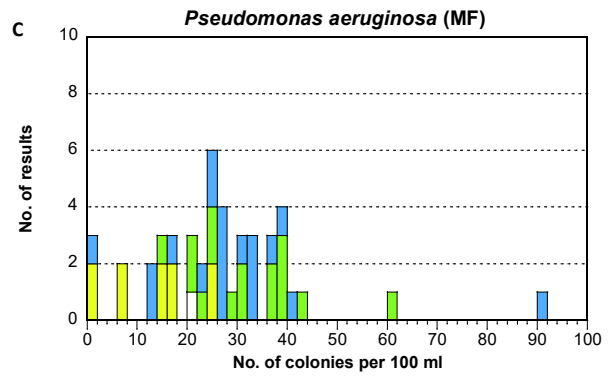
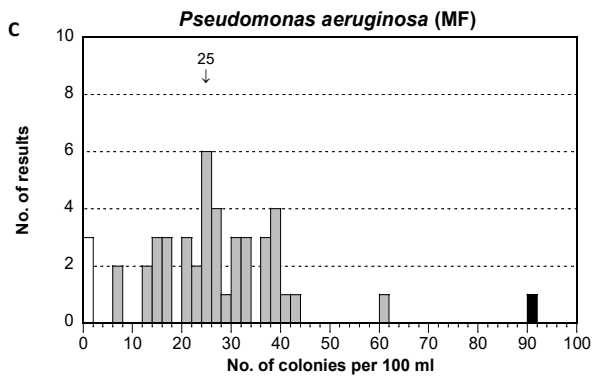
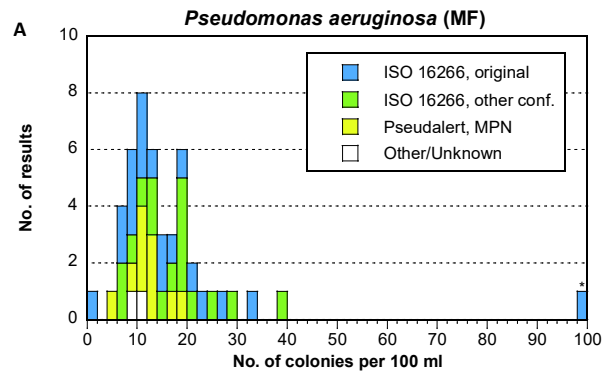
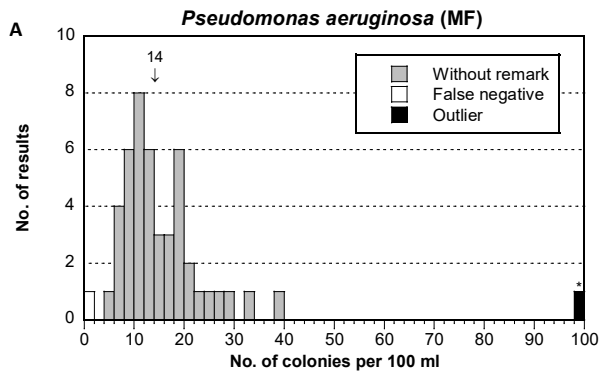
**Table 6.** Results from analysis of *Pseudomonas aeruginosa*.

Medium	Sample A							Sample B							Sample C						
	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>
All results	47	45	14	26	1	0	1	46	46	0	–	0	–	–	46	42	25	25	3	0	1
ISO 16266 <sup>1</sup>	19	17	14	26	1	0	1	19	19	0	–	0	–	–	19	17	27	16	1	0	1
ISO 16266, mod. <sup>2</sup>	16	16	16	24	0	0	0	16	16	0	–	0	–	–	16	16	31	17	0	0	0
Pseudalert®, MPN	10	10	11	19	0	0	0	10	10	0	–	0	–	–	10	8	15	25	2	0	0
Other/Unknown	2	2	–	–	0	0	0	1	1	–	–	0	–	–	1	1	–	–	0	0	0

For “All results”, m = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods, m = mean value for the particular method, with outliers and false results excluded.

<sup>1</sup> Modification not stated for confirmation

<sup>2</sup> Alternative confirmation performed, e.g. Maldi-TOF, API, phenanthroline test



# Culturable microorganisms, 72 hours incubation at $22 \pm 2$ °C

## Sample A

The strains of *L. plantarum* and *S. saprophyticus* were the main target organisms.

In total, 77 participants reported results. Three low and two high outliers were reported.

## Sample B

The strains of *K. pneumoniae*, *E. aerogenes* and *E. hirae* were the main target organism. The strain of *S. capitis* will not grow at  $22 \pm 2$  °C.

In total, 77 participants reported results. One false negative result, as well as one low and three high outliers were reported.

## Sample C

The strain of *P. fluorescens* was the main target organism.

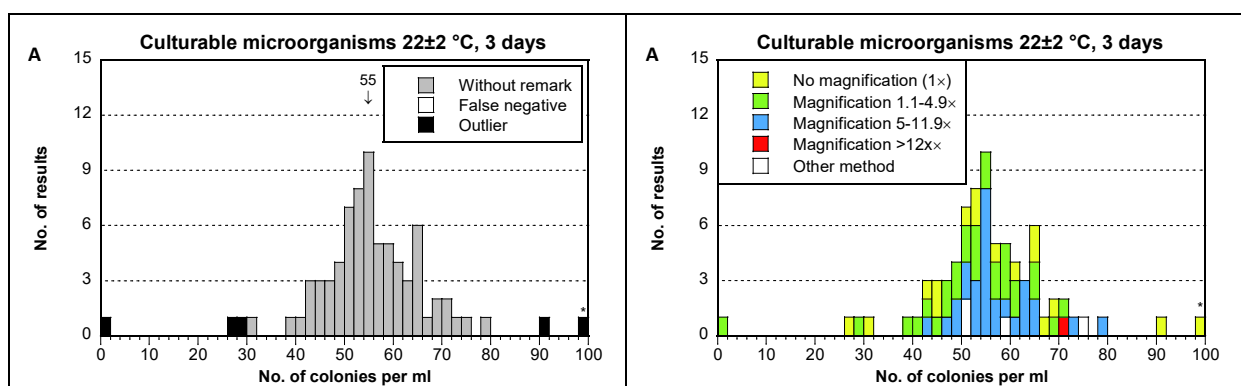
In total, 77 participants reported results. One high outlier was reported.

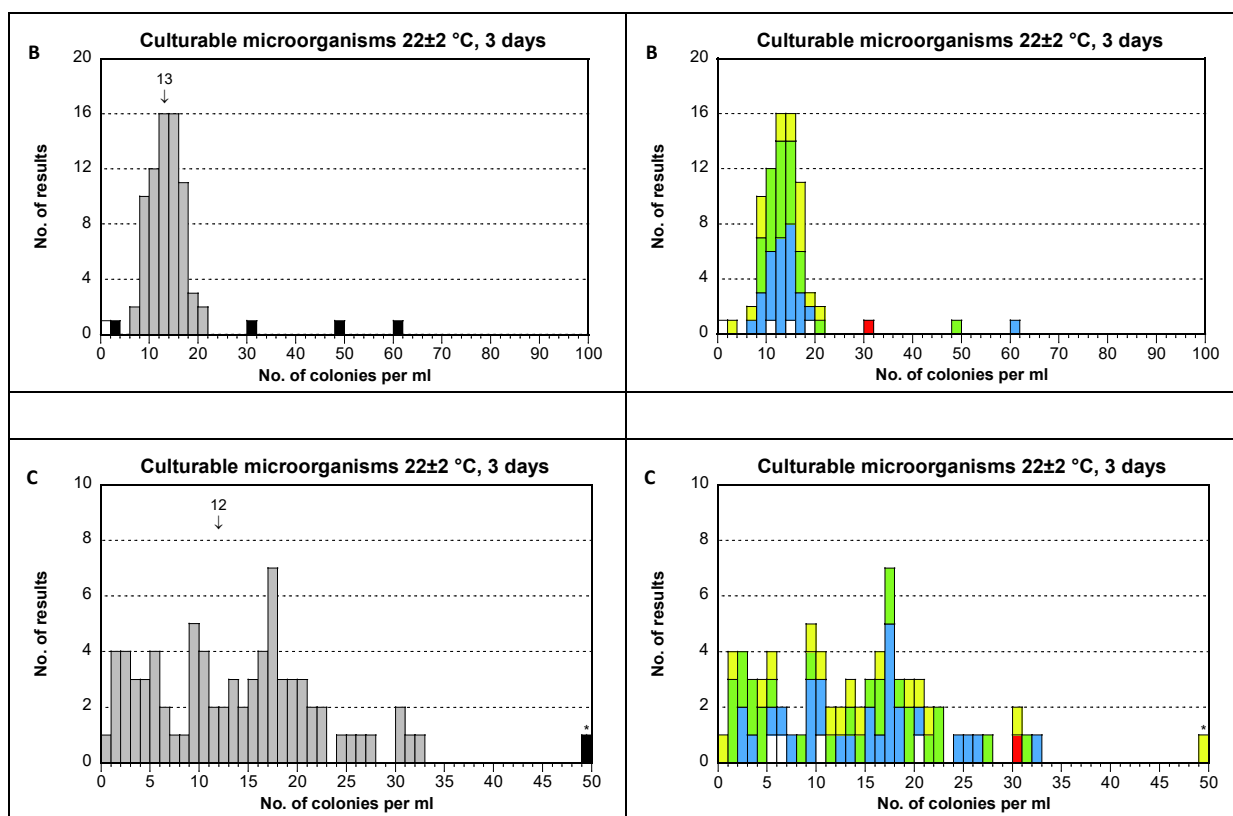
The CV was very large. Similar dispersions have been observed in several previous PT rounds for this strain and even detection of <1 colonies is considered acceptable.

## General remarks

A total of 73 of the 77 participants followed EN ISO 6222:1999, inoculating YeA by pour-plate method. Of these, nine used PCA instead.

The group Other/Unknown in the table includes use of Petrifilm™ and YeA in conjunction with other standards.





**Table 7.** Results from analysis of culturable microorganisms, 72 hours incubation at 22±2 °C.

	Sample A							Sample B							Sample C							
	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	
<b>All results</b>	<b>77</b>	<b>72</b>	<b>55</b>	<b>9</b>	<b>0</b>	<b>3</b>	<b>2</b>	<b>77</b>	<b>72</b>	<b>13</b>	<b>15</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>77</b>	<b>76</b>	<b>12</b>	<b>40</b>	<b>0</b>	<b>0</b>	<b>1</b>	
EN ISO 6222	73	68	55	8	0	3	2	73	69	13	13	0	1	3	73	72	11	38	0	0	1	
Medium																						
Yeast extract Agar	65	63	55	8	0	1	1	65	61	13	13	0	1	3	65	64	12	34	0	0	1	
“Plate count Agar”	8	5	55	9	0	2	1	8	8	13	14	0	0	0	8	8	6	70	0	0	0	
Magnification																						
None	16	13	53	11	0	1	2	16	15	13	15	0	1	0	16	15	10	44	0	0	1	
1.1-4.9x	28	26	54	8	0	2	0	28	27	13	11	0	0	1	28	28	10	44	0	0	0	
5-11.9x	28	28	56	7	0	0	0	28	27	12	12	0	0	1	28	28	13	30	0	0	0	
>12x	1	1	-	-	0	0	0	1	0	-	-	0	0	1	1	1	-	-	0	0	0	
Other method	4	4	-	-	0	0	0	4	3	-	-	1	0	0	4	4	-	-	0	0	0	

For “All results”, m = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods, m = mean value for the particular method, with outliers and false results excluded.

# Culturable microorganisms, 48 hours incubation at 36 ± 2 °C

## Sample A

The strains of *L. plantarum* and *S. saprophyticus* were the main target organisms.

In total, 60 participants reported results.

## Sample B

The strain of *S. capitis* was the main target organism. The strains of *K. pneumoniae*, *E. aerogenes* and *E. hirae* also grow as culturable microorganisms, but in low numbers.

In total, 60 participants reported results. One false negative result and three low outliers were reported.

## Sample C

The few colonies that might occur originate from *E. coli*, *A. hydrophila* and *P. aeruginosa*. The strain of *P. fluorescens* will not grow at 36 ± 2 °C.

In total, 60 participants reported results. Two high outliers were reported.

## General remarks

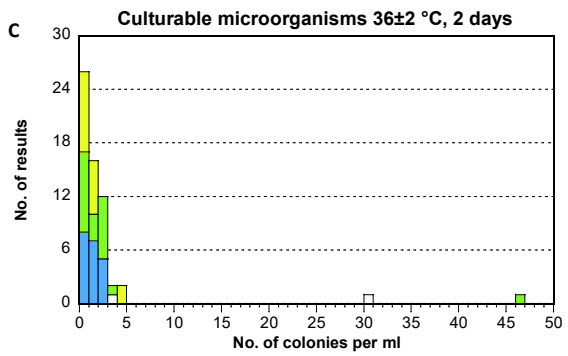
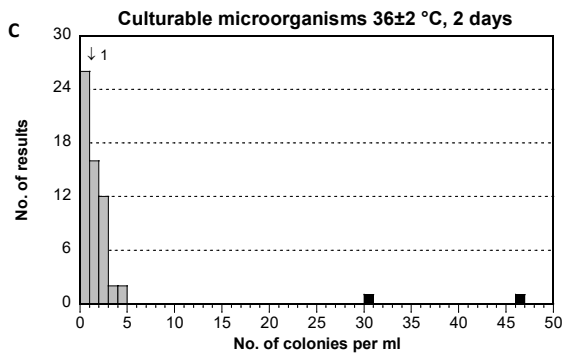
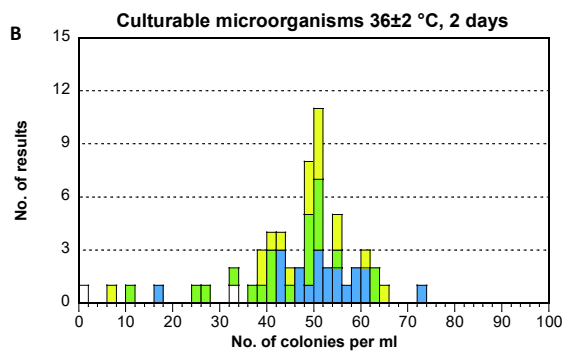
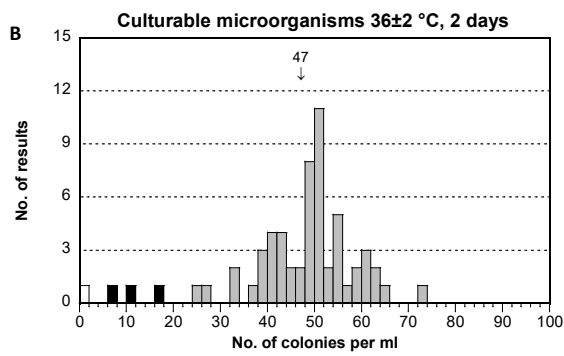
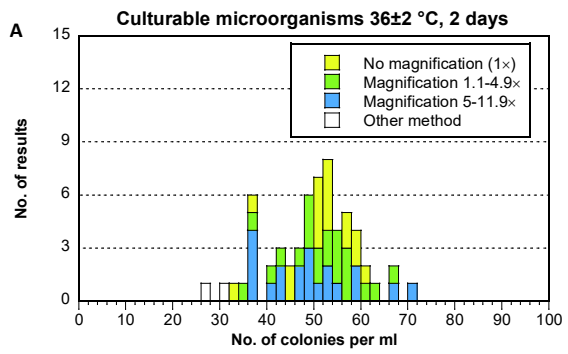
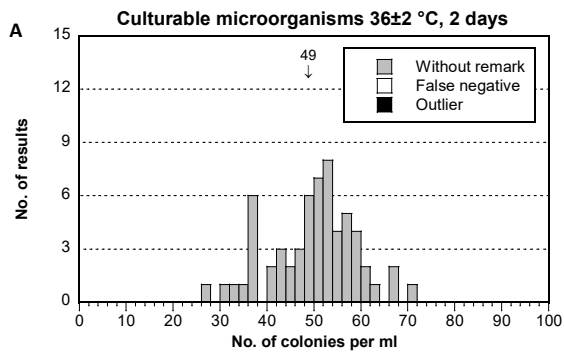
Most participants (97 %) followed EN ISO 6222:1999. Of these, four reported the use of PCA. Both participants in the group Other/Unknown followed Standard Methods [2]. The very high CV of 107 % in sample C is not statistically relevant, as it is mainly a consequence of the very low concentration of target organisms.

**Table 8.** Results from analysis of Culturable microorganisms, 48 hours incubation at 36±2 °C

	Sample A							Sample B							Sample C						
	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>	N	n	m	CV	F	<	>
<b>All results</b>	<b>60</b>	<b>60</b>	<b>49</b>	<b>9</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>60</b>	<b>56</b>	<b>47</b>	<b>10</b>	<b>1</b>	<b>3</b>	<b>0</b>	<b>60</b>	<b>58</b>	<b>1</b>	<b>107</b>	<b>0</b>	<b>0</b>	<b>2</b>
EN ISO 6222	58	58	50	9	0	0	0	58	55	48	10	0	3	0	58	57	0	98	0	0	1
Medium																					
Yeast extract Agar	54	54	50	9	0	0	0	54	51	48	9	0	3	0	54	53	0	104	0	0	1
“Plate count Agar”	4	4	-	-	0	0	0	4	4	-	-	0	0	0	4	4	-	-	0	0	0
Magnification																					
None	17	17	50	8	0	0	0	17	16	49	7	0	1	0	17	17	0	121	0	0	0
1.1-4.9x	21	21	51	8	0	0	0	21	20	44	12	0	1	0	21	20	1	96	0	0	1
5-11.9x	20	20	48	10	0	0	0	20	19	52	7	0	1	0	20	20	0	87	0	0	0
Other method	2	2	-	-	0	0	0	2	1	-	-	1	0	0	2	1	-	-	0	0	1



For “All results”,  $m$  = robust  $m_{PT}$  re-transformed to the cfu scale. For individual methods,  $m$  = mean value for the particular method, with outliers and false results excluded.



# Outcome of the results of individual participants - assessment

## Reporting and evaluation of results

The results of all participants are listed in Annex 1, together with the minimum and maximum accepted values for each analytical parameter. Results that received a remark (false results and outliers) are highlighted and shown with bold font.

Participants are not grouped or ranked based on their results. The performance of an individual participant can be broadly assessed by the numbers of outliers and false results, and by the z-scores.

Information on the results processing and recommendations for follow-up work are given in the Scheme Protocol [3].

Samples for follow-up analyses can be ordered at: [www.livsmedelsverket.se/en/PT-extra](http://www.livsmedelsverket.se/en/PT-extra)

## Box plots and numbers of deviating results for each participant

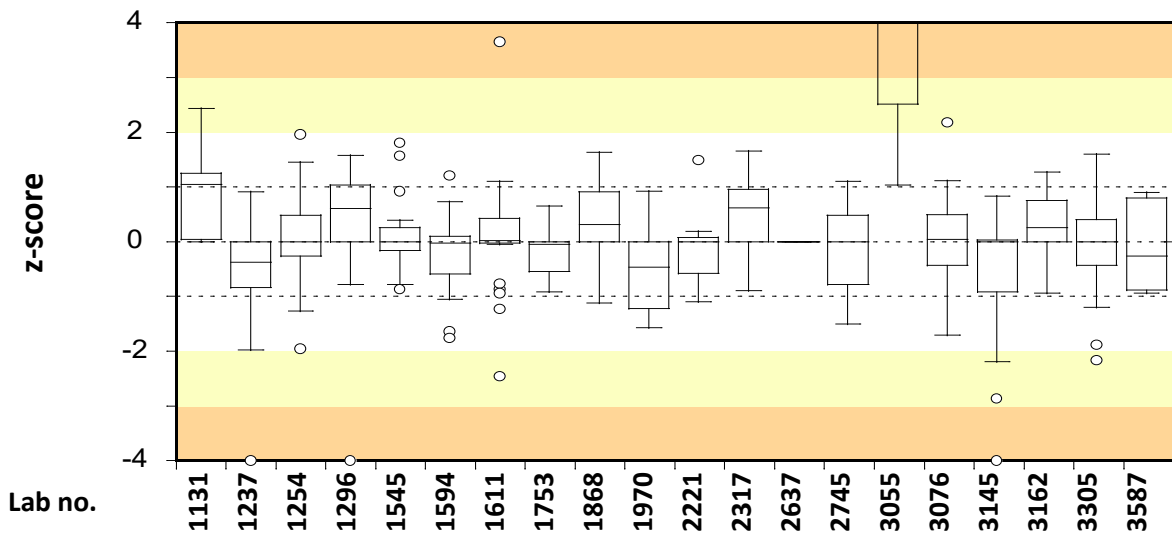
Box plots are based on the z-scores listed in Annex 2, and give a comprehensive view of the performance of each participant. The range of z-scores is indicated by the size of the box and, for most participants, by lines and/or circles above and beneath the box. A small range of values, centred around zero, indicates that the results of the individual participant are in general close to  $m_{PT}$  for the different analyses. For each participant, the number of false results and outliers are also listed in the tables below the box plots.

Outliers are included in the figures after being calculated to z-scores in the same way as for other results. Correct negative results for quantitative analyses without target organism are given a z-score of 0. False results do not generate any z-scores, and are not included in “No. of results”.

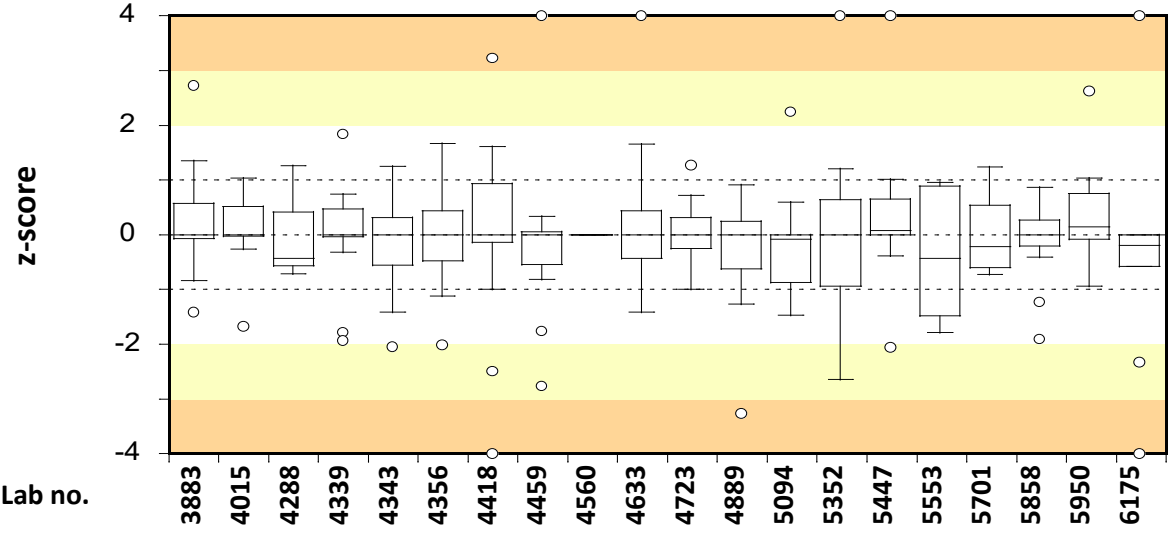
The participant’s median value is illustrated by a horizontal line in the box. Each box includes 50 % of a participant’s results (25 % of the results above the median and 25 % of the results below the median). The remaining 50 % are illustrated by lines and circles outside the box. A circle is for technical reasons shown in the plot when a value deviates to certain degree\* from the other values. This does not by itself indicate that the value is an outlier.

Z-scores  $>+4$  and  $<-4$  are positioned at  $+4$  and  $-4$ , respectively, in the plot. The background is divided by lines and shaded fields to simplify identifying the range in which the results are located.

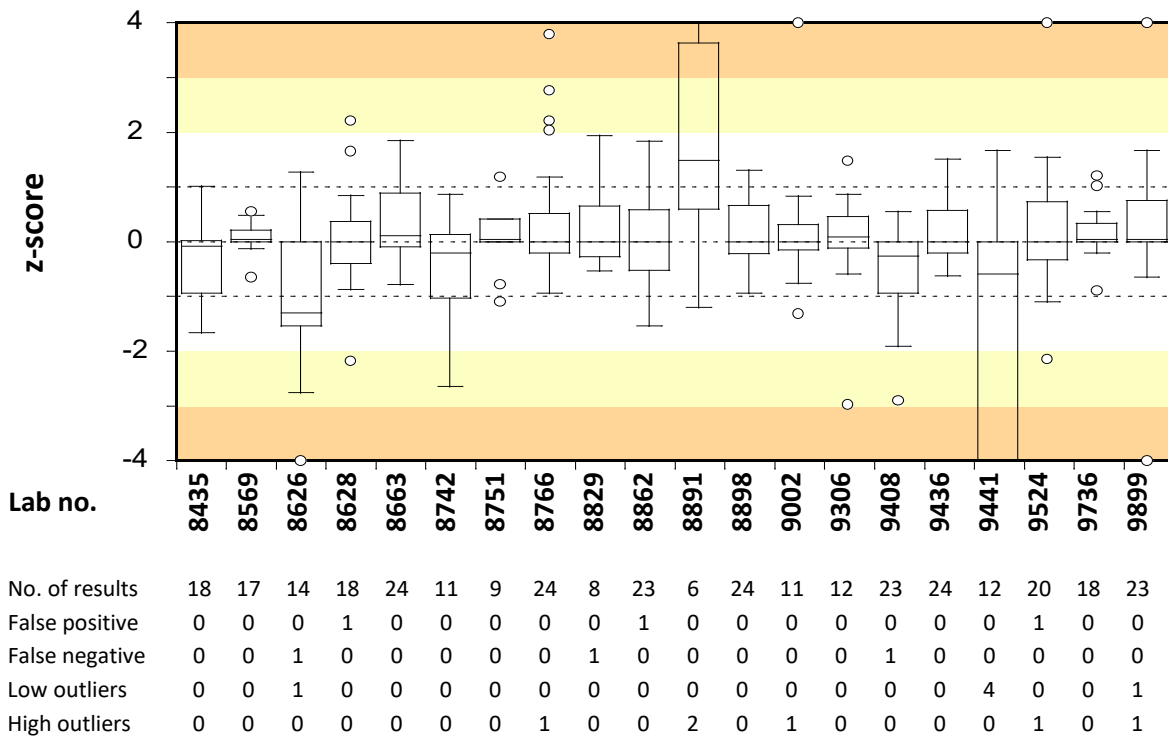
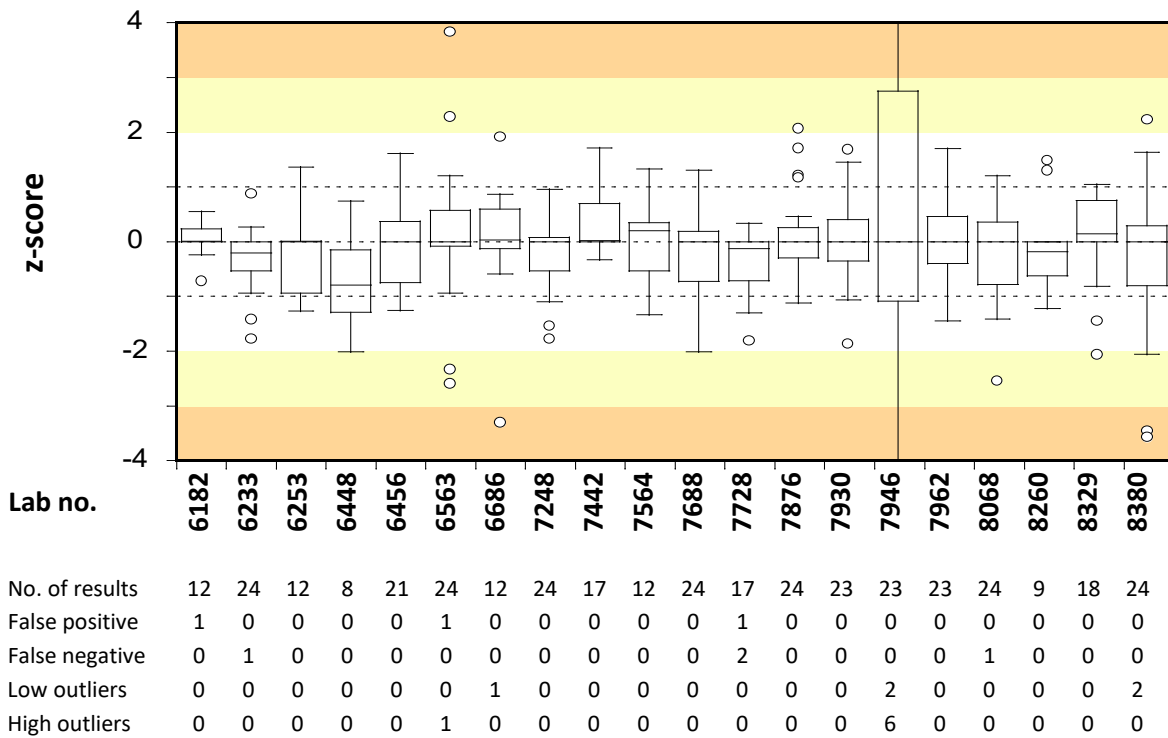
\*  $< [\text{lowest value in the box} - 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})]$   
or  
 $> [\text{highest value in the box} + 1,5 \times (\text{highest value in the box} - \text{lowest value in the box})].$

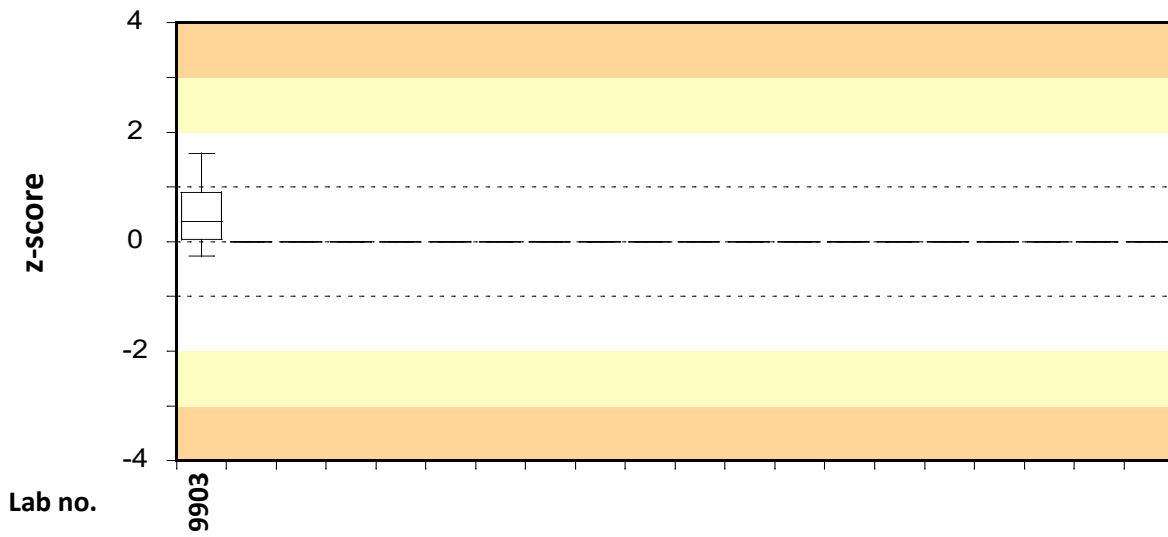


No. of results	9	23	24	15	24	24	24	18	17	17	11	17	0	9	3	9	18	18	24	6
False positive	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
High outliers	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0



No. of results	23	12	3	24	18	24	23	11	0	21	12	23	14	17	18	6	9	17	18	11
False positive	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
False negative	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
Low outliers	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
High outliers	0	0	0	0	0	0	1	1	0	1	0	0	0	1	2	0	0	0	0	1





No. of results	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False positive	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
False negative	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Low outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
High outliers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

# Test material and quality control

## Test material

Each participant received three samples with freeze-dried microorganisms, designated 1–3. The test material was freeze-dried in 0.5 ml portions in glass vials, as described by Peterz and Steneryd [4]. Before analysing the samples, the contents of each vial should be reconstituted in 800 ml of sterile diluent. The microorganism content of the samples and the concentrations determined at the Swedish Food Agency are listed in the table below.

**Table 9.** Microorganisms in the samples.

Sample	Microorganism	Strain			
		SLV no. <sup>1</sup>	Origin	Reference <sup>2</sup>	cfu/100 ml <sup>3</sup>
A	<i>Escherichia coli</i>	SLV-084	Drinking water	-	13
	<i>Klebsiella pneumoniae</i>	SLV-186	Vegetarian kebab	CCUG 45102	13
	<i>Lactobacillus plantarum</i>	SLV-475	-	CCUG 30503	54*
	<i>Pseudomonas aeruginosa</i>	SLV-453	-	CCUG 551	30
	<i>Staphylococcus saprophyticus</i>	SLV-013	-	CCUG 45100	-
B	<i>Klebsiella pneumoniae</i>	SLV-537	-	-	330
	<i>Enterobacter aerogenes</i>	SLV-099	-	ATCC 13 048	190
	<i>Enterococcus hirae</i>	SLV-536	Water	CCUG 46536	750
	<i>Burkholderia cepacia</i>	SLV-042	-	-	28
	<i>Staphylococcus capitis</i>	SLV-463	-	CCUG 35173	41*
C	<i>Escherichia coli</i>	SLV-295	Water	-	30
	<i>Aeromonas hydrophila</i>	SLV-081	Drinking water	CCUG 45103	16
	<i>Pseudomonas aeruginosa</i>	SLV-569	-	-	49
	<i>Pseudomonas fluorescens</i>	SLV-535	-	CCUG 45106	26*

<sup>1</sup> Internal strain identification no. at the Swedish Food Agency

<sup>2</sup> Culture collection. ATCC: American Type Culture Collection, CCUG: Culture Collection University of Gothenburg

<sup>3</sup> cfu = colony forming units; \* indicates cfu per ml

## Quality control of the samples

In order to allow comparison of the freeze-dried samples, it is essential to have aliquots of homogeneous test material and equal volume in all vials. Quality control is performed on 10 randomly chosen vials in conjunction with manufacturing of the samples or on 5 vials if the sample mixture is homogenous but the last quality control was performed more than 6 months ago. Homogeneity of a test material is approved if, for each analysis, the values obtained for the test for “Index of dispersion” between vials ( $I_2$ ) and the test for reproducibility (T) do not simultaneously exceed 2.0. (For definitions of  $I_2$ , and T, see references [5] and [6] respectively.)

**Table 10.** Concentration mean (m),  $I_2$  and T values from the quality control of the sample mixtures; m is expressed in cfu (colony forming units) per 100 ml of sample for MF methods and per 1 ml for pour plate methods.

Analysis and method	A <sup>1</sup>			B <sup>1</sup>			C <sup>1</sup>		
	m	$I_2$	T	m	$I_2$	T	m	$I_2$	T
Coliform bacteria (MF) EN ISO 9308-1:2014	26	0.75	1.40	53 <sup>2</sup>	0.16	1.12	50	1.73	1.44
Suspected thermotolerant colif. bact. (MF) m-FC Agar, 44 °C according to SS 028167	20	0.73	1.48	48 <sup>2</sup>	0.44	1.21	24	1.25	1.58
<i>Escherichia coli</i> (MF) EN ISO 9308-1:2014	13	0.79	1.59	-	-	-	32	<b>2.42</b>	1.72
<i>Intestinal enterococci</i> (MF) SS-EN ISO 7899-2:2000	-	-	-	75 <sup>2</sup>	0.77	1.22	-	-	-
<i>Pseudomonas aeruginosa</i> (MF) SS-EN ISO 16288:2008	29	1.30	1.50	-	-	-	49	1.95	1.53
Culturable microorg., 48 h 37 °C (pour plate) SS-EN ISO 6222:1999	47	1.67	1.48	54	0.00	1.02	1	<b>2.38<sup>3</sup></b>	<sup>-3</sup>
Culturable microorg., 72 h 22 °C (pour plate) SS-EN ISO 6222:1999	53	1.00	1.31	11	0.36	1.42	27	<b>2.72</b>	1.81

– No target organism or no value

<sup>1</sup> n = 5 vials analysed in duplicate

<sup>2</sup> Determined for the volume 10 ml

<sup>3</sup> Concentration too low for statistical measures

# References

1. ISO 13528:2015 Statistical methods for use in proficiency testing by interlaboratory comparison.
2. Standard Methods for the Examination of Water and Wastewater, [www.standardmethods.org](http://www.standardmethods.org)
3. Anonymous, 2018. Protocol. Microbiology. Drinking water & Food, Swedish Food Agency.
4. Peterz, M., Steneryd. A.C. 1993. Freeze-dried mixed cultures as reference samples in quantitative and qualitative microbiological examinations of food. *Journal of Applied Bacteriology*. 74:143–148.
5. Heisterkamp, S.H., Hoekstra, J.A., van Strijp-Lockefeer, N.G.W.M., Havelaar, A.H., Mooijman, K.A., in't Veld, P.H., Notermans, S.H.W., Maier, E.A. ; Griepink, B. 1993. Statistical analysis of certification trials for microbiological reference materials. Luxembourg: Commission of the European Communities, Report EUR 15008 EN.
6. Mooijman, K.M., During, M. & Nagelkerke, N.J.D. 2003. MICROCRM: Preparation and control of batches of microbiological materials consisting of capsules. RIVM report 250935001/2003. RIVM, Bilthoven, Holland.



# Annex 1. Results of the participating laboratories

Lab no.	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria (MPN)			<i>E. coli</i> (MPN)			Susp. intestinal enterococci			Intestinal enterococci			Susp. <i>Pseudomonas aeruginosa</i>			<i>Pseudomonas aeruginosa</i>			Culturable microorganisms, 22 °C (cfu/ml)			Culturable microorganisms, 36 °C (cfu/ml)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	-	-	-	-	-	-	-	-	-	-	-	39	649	31	14	0	0	-	-	-	-	-	-	-	-	-	-	72	13	24	-	-	-			
1237	-	-	-	20	350	13	-	-	-	8	<1	<1	21	461	19	10	<1	<1	-	-	-	<1	80	<1	-	-	7	<1	15	64	9	13	53	44	1	
1254	-	-	-	19	390	37	-	-	-	10	0	37	15	460	28	8	0	0	-	-	-	0	850	0	-	-	12	0	30	68	16	10	52	50	1	
1296	-	-	-	29	500	20	-	-	-	17	0	1	-	-	-	-	-	-	-	-	-	42	750	0	15	0	32	-	-	-	71	15	19	57	48	3
1545	21	610	36	21	610	24	21	410	24	8	0	24	25	457	21	12	0	0	4000	790	0	0	790	0	29	130	37	29	0	37	55	14	5	48	49	1
1594	-	-	-	20	505	31	26	250	17	9	0	31	19	450	20	7	0	0	-	-	-	0	735	0	-	-	19	0	29	57	12	1	45	42	0	
1611	24	530	25	24	530	25	24	293	22	15	0	25	27	291	28	12	0	0	0	1270	0	0	1270	0	15	0	27	15	0	27	47	14	3	60	40	0
1753	-	-	-	-	-	-	-	-	-	-	-	-	22	574	22	9	0	0	0	680	0	0	680	0	-	-	10	0	15	54	13	9	42	54	1	
1868	-	-	-	30	560	31	-	-	-	16	0	0	29	478	24	18	0	0	0	780	0	0	780	0	19	0	28	-	-	-	64	9	17	-	-	-
1970	20	380	19	20	380	19	20	380	19	7	0	0	-	-	-	-	-	-	0	860	0	0	860	0	6	0	12	6	0	12	41	12	2	43	36	1
2221	-	-	-	-	-	-	-	-	-	19	<1	<1	-	-	-	-	-	-	-	-	-	<1	760	<1	-	-	7	<1	22	-	-	-	51	38	<1	
2317	-	-	-	28	380	35	-	-	-	16	0	0	-	-	-	-	-	-	-	-	-	0	730	0	-	-	8	0	33	61	18	19	59	65	1	
2637	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2745	14	370	29	14	370	29	14	370	29	8	0	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	66	8	14	-	-	-	
3055	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	194	17	164	-	-	-	
3076	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33	0	40	51	13	17	50	32	0	
3145	-	-	-	-	-	-	-	-	-	-	-	-	14	579	26	9	0	0	-	-	-	0	727	0	-	-	8	0	25	31	12	21	50	6	0	
3162	-	-	-	-	-	-	-	-	-	-	-	-	31	548	26	16	0	0	5800	791	0	0	791	0	16	0	25	16	0	25	49	17	27	56	55	0
3305	32	470	16	19	470	12	-	-	-	11	<1	12	30	690	19	13	<1	<1	4800	690	<1	<1	690	<1	11	16	30	11	<1	30	47	17	22	45	55	1
3587	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	53	16	9	58	39	<1	
3883	22	450	53	22	450	-	-	-	-	10	<1	20	20	580	42	11	<1	<1	<1	920	<1	<1	920	<1	19	<1	31	19	<1	31	54	16	17	37	51	2
4015	-	-	-	-	-	-	-	-	-	-	-	-	24	570	28	11	0	0	5000	626	1	0	545	0	-	-	-	-	-	59	17	17	-	-	-	
4288	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	51	18	6	-	-	-	
4339	-	-	-	25	510	24	25	390	26	10	0	24	27	345	36	14	0	0	0	850	0	0	517	0	-	-	13	0	0	61	15	9	50	49	1	
4343	-	-	-	-	-	-	-	-	-	-	-	-	23	649	21	6	0	0	5100	855	0	0	855	0	-	-	13	0	16	60	13	15	37	42	1	
4356	20	370	31	17	370	25	23	370	25	4	0	25	34	548	29	16	0	0	0	760	0	0	760	0	20	0	20	20	0	9	5	48	39	2		
4418	0	390	29	0	390	29	21	260	29	17	0	29	33	540	3	0	0	13	0	621	0	0	621	0	10	0	23	10	0	23	90	17	0	53	51	4
4459	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	707	0	-	-	-	-	-	56	10	1	48	24	1	
4560	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4633	-	-	-	25	382	26	13	305	22	20	0	26	20	377	22	15	0	0	-	-	-	0	736	0	-	-	-	-	-	51	49	11	55	45	0	
4723	-	-	-	-	-	-	-	-	-	-	-	-	26	461	20	11	0	0	5364	909	0	0	909	0	-	-	-	-	-	62	12	15	-	-	-	
4889	-	-	-	26	460	25	-	-	-	8	0	0	10	610	27	8	0	0	-	-	-	0	790	0	-	-	9	0	27	56	11	4	56	48	0	
5094	-	-	-	26	362	27	-	-	-	11	0	0	-	-	-	-	-	-	-	-	-	0	726	0	-	-	-	-	47	8	2	42	72	0		
5352	-	-	-	9	970	19	7	0	15	3	0	0	-	-	-	-	-	-	-	-	-	0	900	0	-	-	10	0	33	48	10	2	40	60	2	
5447	-	-	-	5700	1100	27	-	-	-	11	0	27	-	-	-	-	-	-	-	-	-	19	750	0	-	-	11	0	6	65	16	11	50	48	1	
5553	-	-	-	28	340	14	-	-	-	16	0	14	-	-	-	-	-	-	0	580	0	-	-	-	9	-	0	-	-	-	-	-	-	-		
5701	-	-	-	18	570	21	-	-	-	12	70	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	53	12	22	-	-	-		
5858	23	385	32	23	385	32	-	-	-	12	<1	<1	-	-	-	-	-	-	>2000	520	<1	0	520	<1	13	12	20	13	0	20	53	16	3	55	50	2
5950	-	-	-	24	464	22	17	291	26	10	0	22	-	-	-	-	-	-	34	845	0	0	845	0	38	8	38	38	0	38	49	17	20	52	54	0
6175	-	-	-	-	-	-	-	-	-	-	-	-	24	36	>200	10	0	0	-	-	-	-	-	-	-	-	-	-	50	<10	10	30	<10	30	-	-
6182	-	-	-	-	-	-	-	-	-	-	-	-	23	525	27	12	0	0	193	803	0	37	803	0	-	-	-	-	55	15	6	-	-	-		
6233	21	430	24	21	430	21	-	-	-	12	0	21	20	489	24	15	0	0	0	700	0	0	700	0	10	0	0	4	0	0	53	10	8	37	50	0
6253	-	-	-	-	-	-	-	-	-	-	-	-	19	430	33	10	0	0	-	-	-	0	590	0	-	-	-	-	45	14	12	-	-	-		
6448	-	-	-	13	510	20	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	120	-	-	-	-	9	-	59	10	5	-	-	-		
6456	-	-	-	16	590	38	-	-	-	7	0	18	25	406	21	12	0	0	-	-	-	0	591	0	-	-	-	-	68	11	13	56	51	4		
6563	22	490	10	22	490	10	22	490	10	9	<1	10	22	572	25	13	<1	<1	35	1300	<1	35	1300	<1	18	<1	60	18	<1	60	62	15	26	59	46	<1
6686	-	-	-	-	-	-	-	-	-	-	-	-	9.9	453	25.4	13.7	<1	<1	<1	680	<1	-	-	-	-	-	-	-	55	21	19	47	51	2		
7248	21	260	23	21	260	18	9	220	18	13	<1	18	27	517	25	12	<1	<1	<1	570	<1	<1	560	<1	21	<1	26	21	<1	26	54	10	9	53	50	<1
7442	34	430	35	34	430	23	-	-	-	16	0	0	26	595	28	11	0	0	0	830	0	0	830	0	11	0	33	-	-	-	55	18	18	-	-	-
7564	-	-	-	24	550	36	-	-	-	13	0	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	43	9	15	48	49	<1			
7688	18	300	20	18	240	20	-	-	-	18	0	20	24	440	21	13	0	0	0	730	0	0	730	0	10	0	13	10	0	13	58	9	16	62	48	2
7728	-	-	-	15	420	24	-	-	-	10	0	0	-	-	-	-	-	-	-	-	-	0	860	0	-	-	9	0	0	51	7	5	51			

# Annex 1. Results of the participating laboratories

Lab no.	Suspected coliform bacteria (MF)			Coliform bacteria (MF)			Susp. thermotolerant coliform bact. (MF)			<i>E. coli</i> (MF)			Coliform bacteria (MPN)			<i>E. coli</i> (MPN)			Susp. intestinal enterococci			Intestinal enterococci			Susp. <i>Pseudomonas aeruginosa</i>			<i>Pseudomonas aeruginosa</i>			Culturable microorganisms, 22 °C (cfu/ml)			Culturable microorganisms, 36 °C (cfu/ml)			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
8435	25	430	23	17	430	23	22	300	15	6	0	23	-	-	-	-	-	-	1	720	0	0	720	0	6	0	16	6	0	16	65	14	4	35	50	0	
8569	13	414	29	24	414	29	-	-	-	11	0	0	21	558	26	12	0	0	3100	771	0	0	753	0	0	0	0	0	0	0	0	0	0	0	0	0	
8626	15	280	0	15	280	0	3	0	21	15	0	0	-	-	-	-	-	-	12	560	0	0	560	0	-	-	-	-	-	1	10	2	27	33	3		
8628	-	-	-	21	340	21	20	300	25	20	0	25	-	-	-	-	-	-	>100	764	0	37	764	0	10	0	20	10	0	20	78	6	9	54	56	1	
8663	39	520	47	35	520	24	20	260	24	20	0	24	21	460	21	13	0	0	0	910	0	0	910	0	25	0	42	25	0	42	64	11	18	57	41	2	
8742	-	-	-	9	410	32	-	-	-	9	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	53	10	3	36	50	2		
8751	-	-	-	-	-	-	-	-	-	-	-	-	31	406	21	12	0	0	-	-	-	-	-	-	-	-	-	-	-	59	14	16	-	-	-		
8766	21	400	44	21	400	44	24	418	16	11	0	44	31	509	21	28	0	0	3000	700	0	0	700	0	16	0	32	16	0	32	54	12	10	70	52	0	
8829	-	-	-	21	<1	23	-	-	-	9	<1	<1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	15	20	-	-	-			
8862	26	505	31	26	505	19	-	-	-	9	0	0	25	718	28	14	0	0	4600	855	0	15	855	0	-	-	12	0	14	51	11	18	36	46	0		
8891	-	-	-	26	310	5000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	70	30	30	-	-	-	-	-		
8898	23	514	34	23	514	21	-	-	-	13	0	21	29	464	24	14	0	0	4500	874	0	0	874	0	19	0	38	19	0	38	51	14	16	47	61	0	
9002	-	-	-	5700	300	26	-	-	-	10	0	0	-	-	-	-	-	-	-	-	0	810	0	-	-	-	-	-	48	13	21	-	-	-	-	-	
9306	-	-	-	-	-	-	-	-	-	-	-	-	25	488	22	4	0	0	-	-	-	-	-	-	-	-	-	-	59	13	17	53	63	2			
9408	-	-	-	8	360	17	-	-	-	9	<1	<1	21	435	28	12	<1	<1	-	-	-	-	-	-	-	-	<1	<1	25	39	15	1	52	41	<1		
9436	19	500	48	19	500	29	12	273	28	12	0	29	33	450	26	15	0	0	0	718	0	0	718	0	13	15	38	13	0	38	52	12	25	46	43	1	
9441	-	-	-	-	-	-	-	-	-	-	-	-	4	145	3	1	<1	<1	-	-	-	-	-	-	-	-	-	-	55	12	14	66	27	<1			
9524	-	-	-	20	230	22	-	-	-	10	0	0	24	650	30	9	0	0	-	-	-	10	730	0	-	-	-	-	45	12	31	55	63	46	-	-	
9736	-	-	-	-	-	-	-	-	-	-	-	-	25	517	32	12	0	0	4874	748	0	0	748	0	8	0	24	8	0	24	53	15	17	51	58	1	
9899	21	521	48	21	521	31	-	-	-	12	0	0	21	595	34	12	0	0	0	775	0	0	775	0	14	0	25	14	0	25	65	61	12	66	16	1	
9903	-	-	-	28	400	27	-	-	-	13	0	25	-	-	-	-	-	-	-	-	-	26	800	0	-	-	-	26	0	37	57	13	32	58	59	2	
N	28	28	28	57	57	56	22	22	22	57	56	34	50	50	49	51	51	48	38	41	40	60	60	60	29	28	29	47	46	46	77	77	77	60	60	60	
n	28	28	28	53	54	54	22	22	22	55	55	33	46	48	47	46	51	48	38	41	40	52	56	59	29	28	29	45	46	42	72	72	76	60	56	58	
m <sub>PT</sub>	-	-	-	4.714	20.68	5.035	-	-	-	3.358	-	4.748	4.928	22.63	5.005	3.446	-	-	-	-	-	-	27.21	-	-	-	-	-	3.679	-	4.956	7.414	3.585	3.435	7.017	6.877	0.735
s <sub>PT</sub>	-	-	-	0.649	2.571	0.723	-	-	-	0.674	-	0.681	0.541	2.267	0.539	0.488	-	-	-	-	-	-	2.308	-	-	-	-	-	0.948	-	1.220	0.643	0.520	1.378	0.662	0.717	0.785
u <sub>PT</sub>	-	-	-	0.109	0.433	0.123	-	-	-	0.112	-	0.146	0.096	0.401	0.096	0.085	-	-	-	-	-	-	0.372	-	-	-	-	0.173	-	0.225	0.092	0.074	0.196	0.107	0.116	0.127	
CV (%)	-	-	-	14	12	14	-	-	-	20	-	14	11	10	11	14	-	-	-	-	-	8	-	-	-	-	-	26	-	25	9	15	40	9	10	107	
u <sub>rel,mPT</sub> (%)	-	-	-	1.9	1.7	2.0	-	-	-	2.7	-	2.5	1.6	1.4	1.5	2.0	-	-	-	-	-	1.1	-	-	-	-	3.8	-	3.6	1.0	1.7	4.6	1.2	1.3	13.8		
F+	-	-	-	0	0	0	-	-	-	0	1	0	0	0	0	0	0	0	-	-	-	8	0	1	-	-	0	0	0	0	0	0	0	0	0	0	
F-	-	-	-	1	1	1	-	-	-	1	0	0	0	0	0	1	0	0	-	-	-	0	1	0	-	-	1	0	3	0	1	0	0	1	0		
<	-	-	-	0	0	0	-	-	-	0	0	1	3	2	2	1	0	0	-	-	-	0	1	0	-	-	0	0	0	3	1	0	0	3	0		
>	-	-	-	3	2	1	-	-	-	1	0	0	1	0	0	3	0	0	-	-	-	0	2	0	-	-	1	0	1	2	3	1	0	0	2		
Min	0	260	0	0	0	0	3	0	10	0	0	1	4	36	3	0	0	0	0	120	0	0	0	0	0	0	0	0	0	0	0	0	0	27	0	0	
Max	750	680	65	5700	1100	5000	750	568	65	155	70	44	140	718	42	140	0	0	5800	1300	1	42	1300	860	800	130	90	800	0	90	194	61	164	70	72	46	
Med	21	430	31	21	412	24	21	300	23	11	0	24	24	517	26	12	0	0	12	771	0	0	750	0	15	0	27	13	0	25	55	13	13	50	49	1	
m <sub>PT</sub> <sup>2</sup>	-	-	-	22	427	25	-	-	-	11	0	23	24	512	25	12	0	0	-	-	-	0	740	0	-	-	14	0	25	55	13	12	49	47	1		
Lower	0	0	0	7	167	8	0	0	0	1	0	7	11	250	11	3	0	0	0	0	0	0	411	0	0	0	0	1	0	1	30	4	0	25	22	0	
Upper	750	680	65	45	806	52	750	568	65	29	0	47	43	867	44	25	0	0	5800	1300	1	0	1165	0	800	130	90	43	0	75	88	27	58	81	82	10	

N = number of reported results  
n = results without annotation  
Min = lowest reported result  
Max = highest reported result  
Med = median value  
m<sub>PT</sub> = assigned value  
s<sub>PT</sub> = standard deviation  
CV = coefficient of variation  
u<sub>PT</sub> = measurement uncertainty  
u<sub>rel,mPT</sub> = relative standard uncertainty  
F+ = false positive  
F- = false negative  
< = low outlier  
> = high outlier  
Lower = lowest accepted value  
Upper = highest accepted value

- False positive or false negative
- Outside the acceptance limits
- Results "larger than" are not evaluated
- The parameter is not evaluated
- The result not evaluated
- u<sub>PT</sub> > 0,3 s<sub>PT</sub> and/or > 20 % outliers





## Internal and external control for microbiological analyses of food and drinking water

All analytical activities require work of a high standard that is accurately documented. For this purpose, most laboratories carry out some form of internal quality assurance, but the analytical work also needs 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 PT, identical test material is analysed by a number of participants. After reporting of results by the participants, the organiser evaluates the results and compiles them in a report.

### The Swedish Food Agency's PT program offers

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

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

### The Swedish Food Agency's reference material

As a complement to the proficiency testing, but without specific accreditation, the Swedish Food Agency also manufactures a number of reference materials (RM) for internal quality control of food and drinking water microbiological analyses, including pathogens.

For more information, visit our website: [www.livsmedelsverket.se/en/RM-micro](http://www.livsmedelsverket.se/en/RM-micro)