The Characteristics of a Microbiological Food and Drinking water PT Protocol in the Nordic countries

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Why is NFA a PT provider?

- NFA as the central food and drinking water (dw) administration deals with various tasks
  - legislation, inspections, food recommendations, applied research, analyses etc.
- Before accreditation NFA also had to approve laboratories for microbiological analyses in statutory sampling
- NFA developed the PT program *(PT-micro)* during that period, as a tool in the approval process

cont. Why is NFA a PT provider?

- Provided test material for most regulated parameters in Swedish food/dw ordinances
  - microbiological methods were usually explicitly stated
- Adapted later to the similar situation in the other Nordic countries
- NFA continued as PT provider when accreditation took over the approval process
  - had the equipment, experience, routine and staff

NFA work for laboratory support

Advices to laboratories

Meetings for lab. staff

RM – Reference Material
(internal control)

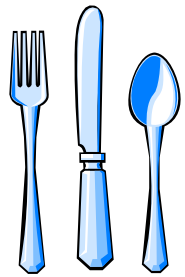
PT – Proficiency Testing
(external control)

NRL activities within EU
(National Reference Laboratory)

Standardisation *(ISO, CEN, NMKL)*

History of the PT-micro program

- Food scheme
  - started for Swedish laboratories 1981
  - Nordic scope 1988
  - now “all” countries are welcome



- Drinking water scheme
  - started for Swedish laboratories 1989
  - Nordic scope 1992
  - now “all” countries are welcome

NFA accredited as PT provider since December 2004

Accreditation Certificate

SWEDAC

Styrelsen för ackreditering och teknisk kontroll
Swedish Board for Accreditation and Conformity Assessment

ACKREDITERINGSBEVIS

ACCREDITATION CERTIFICATE

Livsmedelsverket

har genom beslut den
following the decision on

10 december 2004

ackrediterats som
is accredited as

Arrangör av interlaboratoriva provningsjämförelser inom livsmedelsområdet
Provider of interlaboratory comparisons in food area

och därvid erhållit registreringsnummer
and has been assigned registration number

1457

Styrelsen för ackreditering och teknisk kontroll
Swedish Board for Accreditation and Conformity Assessment

Hans-Eric Holmqvist
Generaldirektör
Director General

Ackrediterat organ har rätt att använda nedanstående märke.
An accredited body is entitled to use the following logotype.

Ackrediteringens omfattning och villkor framgår av ackrediteringsbeslutet.
The scope and conditions of accreditation are specified in the accreditation decision.

- Complying with international documents

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1457
ISO-Guide 43-1

PT-micro work at NFA – all phases

- **Schemes**: Planning & Co-ordination
- **Samples**: Production, Control & Transport
- **Results**: Evaluation & Report

Internet for information exchange **www.slv.se/absint**

Protocol for the program is available there

Kvalifikationsprovningsprogram

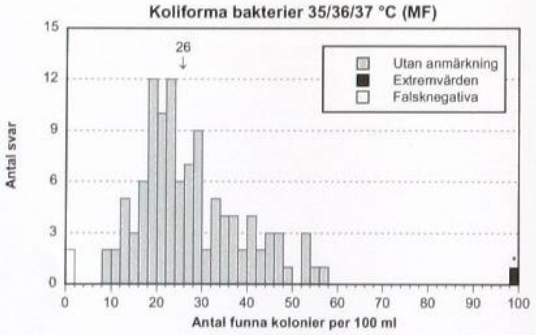
VERKSAMHETSPROTOKOLL

Mikrobiologi

Dricksvatten

&

Livsmedel



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Proficiency Testing Schemes

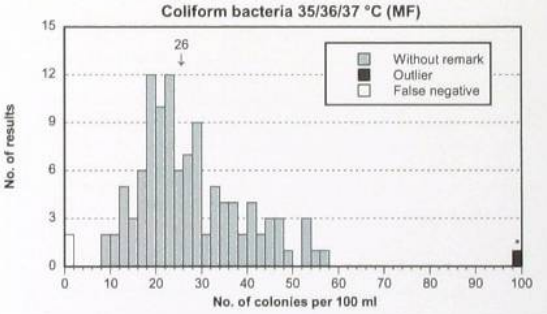
PROTOCOL

Microbiology

Drinking water

&

Food



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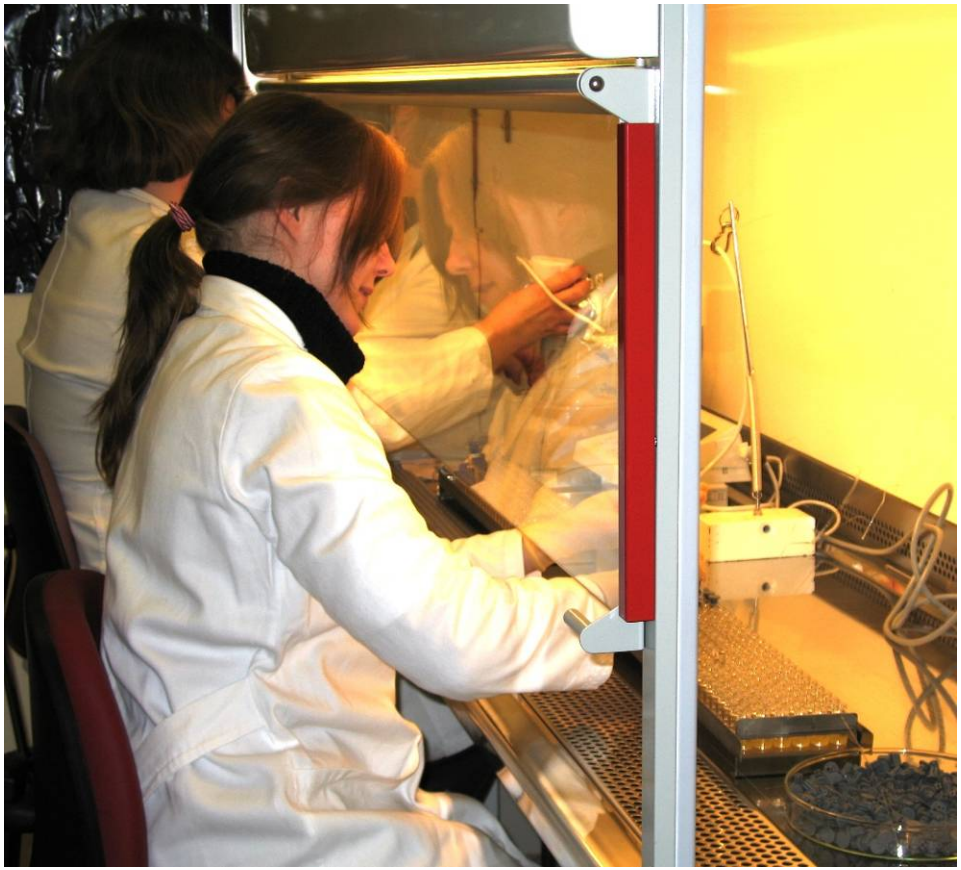
Freeze-drying – our tool

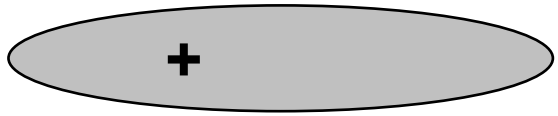


Freeze-dried control material

- **“Matrix free”** mixtures of bacteria/fungi – prepared from pure cultures – in glass vials
- **Simulating** real samples after reconstitution
- **Stable** for long time (often > 2 yrs at −70 °C)
- **Good homogeneity** (due to good survival)
  - testing 10 vials in duplicate
- **Large batches** – up to 2500 vials

Vials with test material

Freeze-dried “matrix free” test items



Advantages

- Mixture of organisms for several parameters
- Good stability
- Good homogeneity
- Produced in large numbers
- Standardised procedure
- Can be used for “spiking”
- Tough and small size – easy transport

Disadvantages

- Need of a good freeze-drier
- Needs reconstitution in liquid
- “Cryoprotectors” for survival – some foam in the sample
- Look quite different from real food samples
- No matrix effects

Evaluation of results – “the work in the dark”

- All reported results compiled in an Appendix
- Results reported back in the same scale as used in reporting from laboratories
  - » calculated results presented in that scale in figures and tables
- No combined scores for several parameters

Transformations – Food microbiology

$$count = 64 \rightarrow \ldots \rightarrow \text{“}count\text{”} = 6.4 \cdot 10^6,\ \log_{10}(\text{“}count\text{”}) \Rightarrow 6.806$$

- often using dilution series due to high numbers
  - “counts” are real cfu* counts calculated per original volume/weight
- $\log_{10}$(“counts”) used for reporting results in/out
  - technicians used to $\log_{10}$ numbers (praxis)
- $\log_{10}$(“counts”) are used for calculations
  - $\log_{10} \Rightarrow$ decent normal distributions directly obtained

** colony forming units*

Transformations – Drinking water microbiology

$$count = 72, \sqrt{72} \Rightarrow 8.485$$

- counts (integers) are used for reporting results
  - technicians used to natural numbers (cfu*) for dw
  - counts usually expressed for the test volume used (100 *or* 1 ml)
  - usually low numbers – **Poisson distribution** prevail
- square root of counts are used for calculations
  - taking the square root ⇒ more normal distribution, usually better transformation than logarithms or none

** colony forming units*

Handling of extreme results

- Extreme results do occur
- Results are often approx. normal distributed
  - after appropriate transformation (*cf*. above)
- Using outlier test for normal distributions
  - the test of Grubbs and Beck, modified by Kelly (1993) as a reduction in SD after removal of 1-2 extreme/s
  - using the test two-sided at the 1%-level
  - used also when a particular distribution is not perfectly normal – an objective tool, fit for our purpose

Robust mean & standard deviation

- False positive results
  - removed without any test
- False negative results
  - removed without any test (high counts; food) *or* a zero result that is a low outlier ("low" counts; dw)
- "Robust" mean (mv) and standard deviation (SD) are obtained with false and extreme results excluded
  - not very deviating from robust values obtained by "Robust statistics" (e.g. Huber's method) when tested

z-scores

- z-scores are calculated by using the current SD of the parameter in a particular round
  - Implications
    - No real comparisons of trends over time possible
    - A “difficult” organism mixture – leading to high SD – will not cause more laboratories than otherwise to obtain extreme z-scores (i.e. $|z|>3$)
  - This is fit for <u>our</u> purpose

cont. z-scores

- Artificial z-scores are calculated in the same way for the outliers (but not from false negatives)
- A box plot is produced for each participant using its z-scores (including the artificial ones)
  - The number of z-scores is given beneath the box plot

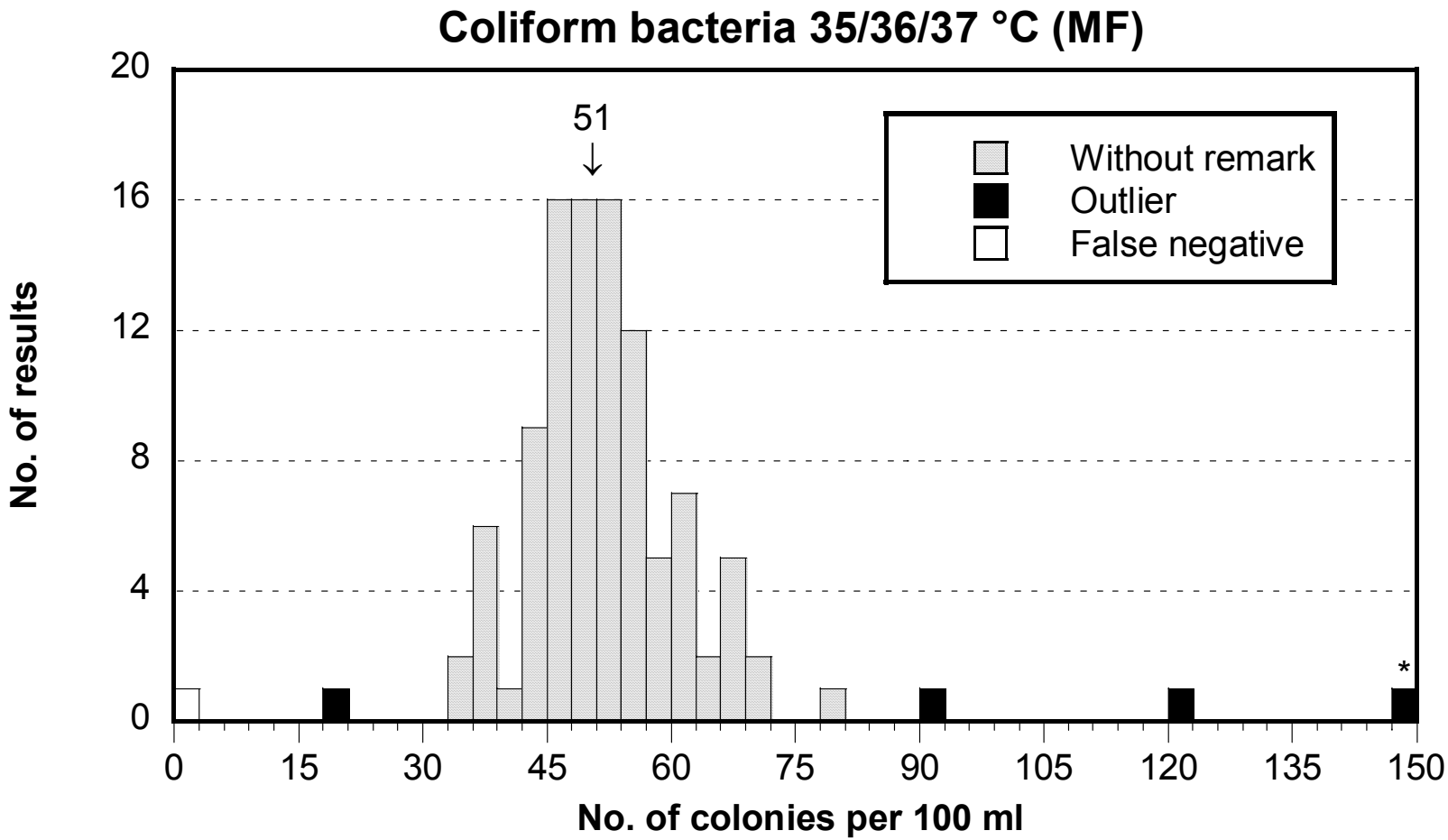
“Visualization” of results – final report

- **All reported values** given in an *Appendix*
  - False results and outliers are shaded and in boldface
- Reported values are **shown** in *histograms*
  - False negative results and outliers differently coloured
- **Summarising** participant results in *box plots*
  - Indicating performance together with numbers of deviating results beneath them – used by assessors
- **Descriptions** of the outcomes of results
  - Discussions about unexpected results

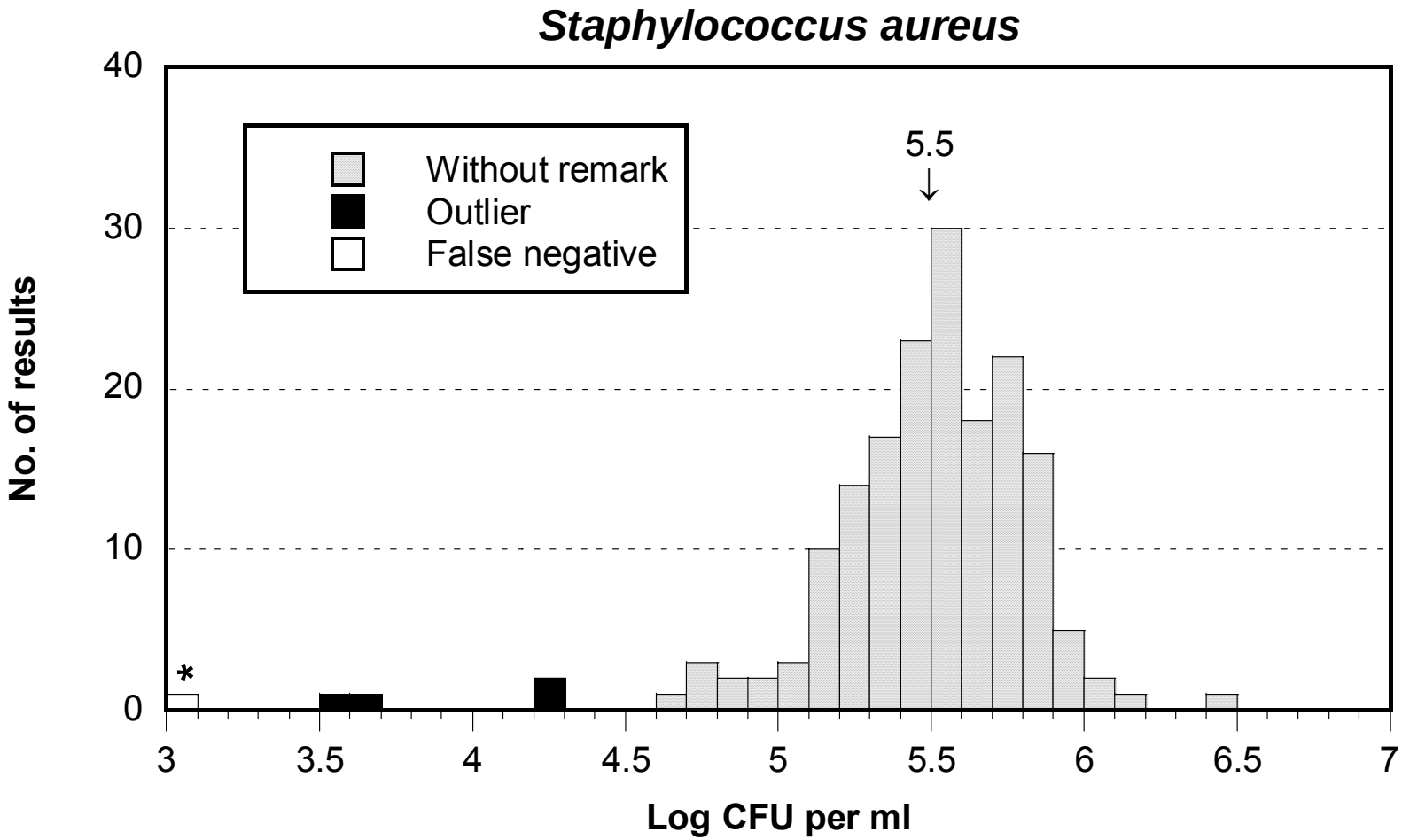
Extract from an Appendix

Lab no.	Sample			Coliform bacteria (MF)			*E. coli* (MF)			*C. perfringens* (MF)			Mould (MF)			Yeast (MF)			Total plate count 22 °C, 3 days		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1131	3	2	1	1527	128	32	1391	0	32	-	-	-	-	-	-	-	-	-	22	46	26
7596	1	2	3	1700	230	12	1100	0	20	8	0	0	-	-	-	-	-	-	25	55	21
7626	2	1	3	1609	82	36	1691	0	20	23	0	0	0	0	260	0	17	**0**	19	38	26
7688	2	3	1	**160**	170	34	**160**	0	34	-	-	-	-	-	-	-	-	-	30	56	27
7720	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7728	2	3	1	2133	147	52	2133	0	52	-	-	-	-	-	-	-	-	-	21	29	18
7876	2	1	3	1490	105	36	1490	<1	36	-	-	-	<1	<1	290	<1	22	82	21	19	14
7885	2	1	3	1480	**0**	40	1330	0	25	3	0	0	-	-	-	-	-	-	17	30	22
7930	2	3	1	2000	280	20	2000	0	20	15	0	0	-	-	-	-	-	-	17	36	32
7962	2	3	1	1900	140	55	1900	0	55	-	-	-	0	0	82	0	13	64	14	58	26
7968	1	2	3	1700	153	12	1700	0	12	-	-	-	-	-	-	-	-	-	18	40	21
7988	2	1	3	1600	90	49	1200	0	27	12	0	0	0	0	482	0	11	**0**	19	25	18
8068	1	2	3	**119**	**1380**	17	**0**	**690**	17	**0**	**11**	0	-	-	-	-	-	-	34	21	14
8255	3	2	1	1920	196	-	1920	0	-	-	-	-	0	0	-	0	22	-	17	60	-
8260	2	1	3	2846	163	38	2846	<1	38	13	<1	<1	-	-	-	-	-	-	25	60	25
8329	2	1	3	1473	127	44	1564	0	34	-	-	-	0	0	282	0	8	54	23	54	21
8380	1	2	3	1460	140	52	1100	0	37	-	-	-	-	-	-	-	-	-	13	54	18
8435	3	2	1	2000	160	28	1800	0	11	18	0	0	-	-	-	-	-	-	21	12	16
8565	2	1	3	1464	185	22	1464	0	22	8	0	0	0	**10**	**0**	0	**0**	**240**	13	27	16
8569	2	1	3	1600	180	28	-	-	-	-	-	-	-	-	-	-	-	-	15	50	22
Median				1640	160	24	1600	0	21	13	0	0	0	0	250	0	13	68	20	45	21
Mean				**1692**	**173**	**26**	**1613**	**0**	**22**	**13**	**0**	**0**	**0**	**0**	**241**	**0**	**13**	**68**	**20**	**43**	**21**
CV (%)				10	25	21	14	-	22	28	-	-	-	-	24	-	20	16	12	20	14

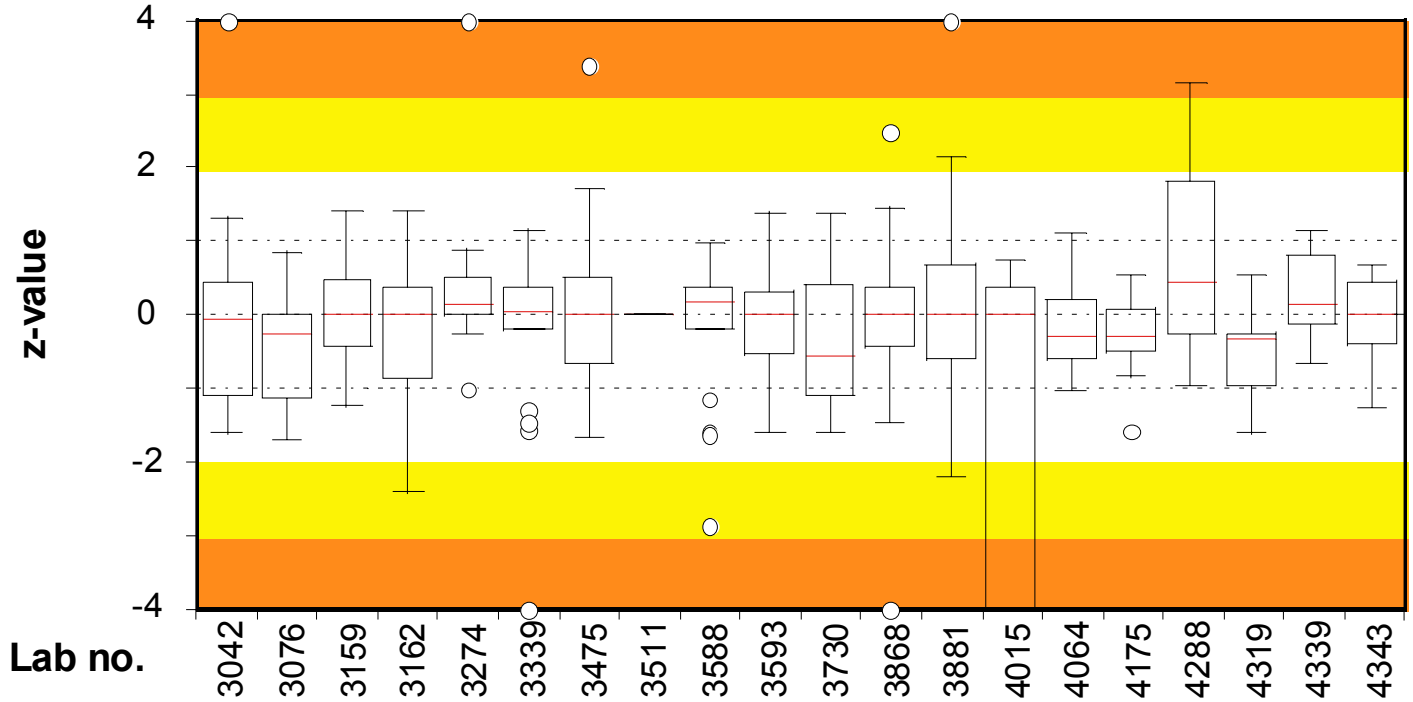
Histogram, Drinking water



Histogram, Food



Box plot, “all” results/laboratory



Lab no.	3042	3076	3159	3162	3274	3339	3475	3511	3588	3593	3730	3868	3881	4015	4064	4175	4288	4319	4339	4343
No. of results	24	9	15	24	23	18	24	-	17	24	3	23	16	18	12	15	3	9	24	24
False positive	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
False negative	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Low outliers	-	-	-	-	-	1	-	-	-	-	-	1	-	5	-	-	-	-	-	-
High outliers	1	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-

Laboratory performance

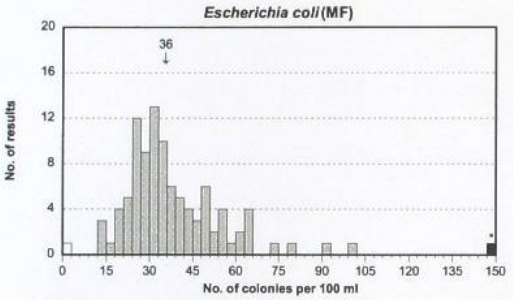
- Numbers of false positives, false negatives, low and high outliers are stated per laboratory, in a table beneath its box plot
- The ***box plot together with the table*** are indicating participant performance
- No other judgement or ranking of the participants are done

Rapport 13 – 2008

Proficiency Testing

Drinking Water Microbiology
2008:1, March

by Tommy Šlapokas, Christina Gunnarsson and Anna Jentzen



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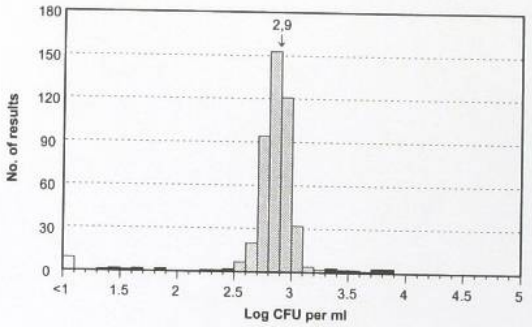
Rapport 25 – 2007

Proficiency Testing

Food Microbiology
– October 2007

by C Normark and K Mykkänen



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Follow-up

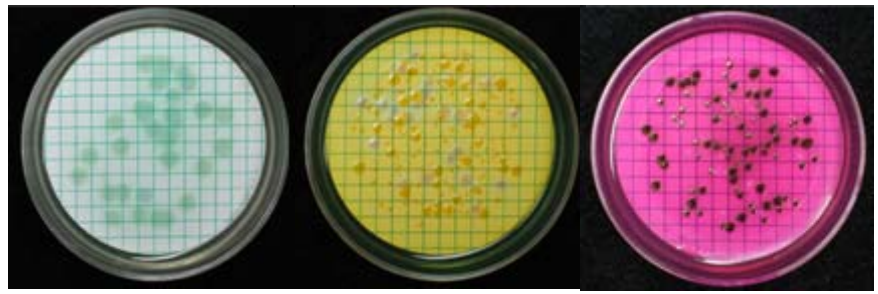
- The participant has the responsibility for their own follow-up
- Test items for follow-up are obtained for free
- Participants may contact NFA for discussions

Why such a low value?

The NFA PT-micro program today

- Food scheme
  - ≈ 280 laboratories
  - 2-4 test items, 3 rounds/year

- Drinking water scheme
  - ≈ 160 laboratories
  - 2-4 test items, 2 rounds/year



Rounds, parameters & test items

- Partly different parameters in the rounds
  - » frequency of most parameters at least once every year
- Test items – mixtures of organisms with known properties for pre-defined purposes
  - » 2-4 (usually 3) vials with 2-6 strains in each
- **Education** is an important purpose for NFA in providing PT schemes

Proficiency testing (PT) of microbiological laboratories

The National Food Administration in Sweden are since many years organising proficiency testing schemes for laboratories performing microbiological analyses in **drinking water** and **food**. By participating the laboratory will get an external evaluation of the skill of the laboratory and its results in relation to the other participating laboratories. All laboratories fulfilling and accepting the conditions and obligations are welcome to participate.

The laboratories have the possibility to record which methods are used and how they are used for the various analyses, respectively, for better evaluation of the obtained results. The information is used for grouping of results for a particular analysis, in order to elucidate possible differences between methods.

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Currently on the agenda - Drinking water scheme

- Mars 2008: Final report is available as pdf after logging in. *(30/06/2008)*
  Printed report can be ordered from kundtjanst@slv.se. Price 100 SEK.
- Follow up samples will be sent at the end of August. *(29/06/2008)*
  Order before August 20.
- Next round: September 2008 *(28/06/2008)*
  Last date for cancellation and registation of new laboratories is August 19.

Currently on the agenda - Food scheme

- April 2008: Final report is available as pdf after logging in. *(30/06/2008)*
  Printed report can be ordered from kundtjanst@slv.se. Price 100 SEK.
- Follow up samples will be sent at the end of August. *(29/06/2008)*
  Order before August 20.
- Next round: October 2008 *(28/06/2008)*
  Last date for cancellation and registation of new laboratories is September 16.

www.slv.se/absint

< Back

Website – www.slv.se/absint

- General info. given on an open part of the website
  - News, time table, content, conditions, costs
  - More comprehensive information in the Protocol (pdf document)
  - A form for application to become a registered participant

- Reporting forms and specific information available for participants only (have to log on)
  - Forms for reporting numerical results and method information
  - Page with recorded values and preliminarily calculated results
  - Final reports and other scheme information
    - *Final reports also available on the NFA public page:* **www.slv.se**

Thank you!

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