4th Microbiology PT Evaluation Workshop within the SADCMET Proficiency Testing Scheme for Water Testing Laboratories

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Summary

A major improvement of the microbiology PT scheme provided by Uganda Bureau of Standards (UNBS) has been made by including expert laboratories to calculate from their results an assigned value for performance assessment. With these results statistics were applied for mathematical evaluation (Algortihm A, ISO 13528). Nevertheless transport times and temperatures still prove to be critical. Even with these difficulties approximately one third of the participating laboratories reported excellent, satisfactory or questionable results for the total plate count sample. At least some of the unsatisfactory results are most probably due to logistical problems but still there seems to be a great need for improvement in some laboratories.

The information reported on the methods used revealed problems with the handling of ISO standards. Therefore training focused on the principles of using international standards as well as on the details of membrane filtration methods. Internal quality control of microbiological membrane filtration methods were discussed.

The opportunity for networking and sharing experiences with other microbiologists was used by all participants. They were really interested in the topics discussed and valued the workshop as helpful for improvement of their laboratory work.

Introduction

This report summarizes the topics and discussions of the evaluation workshop for the 4th microbiological PT for drinking water laboratories provided by UNBS. It is meant to inform all interested laboratories and help with corrective actions. The workshop was held in Port-Louis, Mauritius, following the 4th microbiological PT round in 2011. Previous workshops on preceeding PT rounds have been held at Windhoek (Namibia, 2010), Mahe (Seychelles, 2009) and Kampala (Uganda, 2008). The reports are available from http://www.sadcmet.org. As in the past years the chemistry group met at the same time and location to discuss the evaluation of the chemistry PT provided by Namwater and future sustainability of the PT scheme.

During the workshop the general assembley of the SADCWaterLab Association elected a new project management committee. The new PMC faces the task of impoving networking facilities and cooperation between the laboratories that are members of the association.

Workshop

Participants of the two workshops were welcomed in an opening ceremony by

Mr K. Ramful, Mauritius Standards Bureau (MSB) Director
Ms Kezia Mbwambo, SADCWaterLab Association Chair
Ms Kathrin Wunderlich, PTB
Mr Cader Sayed Hossen, Minister of Industry, Commerce and Consumer Protection, Republic of Mauritius

The workshop program was scheduled to start with the evaluation of the PT but unfortunately this had to be changed as the colleagues from Uganda arrived a day late. In order not to start with the evaluation without the PT provider from Uganda present the

workshop started with training topics instead. Also for chemistry and microbiology group together the reports from local coordinators and participants from the Train of Trainer (ToT) program were given.

After these general topics the participants split up into a chemistry and a microbiology group to have training and PT evaluation according to their respective needs and PT schemes.

On Wednesday the PMC had a meeting and Thursday the SADCWaterLab Association held a general assembly including an election of a new PMC. The secretariat will prepare minutes and make them available on the website.

Participants

The microbiology workshop was attended by 22 participants representing laboratories of the listed countries:

Botswana	1	Lesotho	1	Swaziland	1
Burundi	1	Malawi	1	Tanzania	1
DRC	1	Mauritius	4	Uganda	1
Ethiopia	1	Namibia	2	Zambia	1
Ghana	2	Seychelles	1	Zimbabwe	1
Kenya	1	South Africa	1		

A complete list of participants including email addresses is given in annex 1.

Reports of local coordinators

Local coordinators originally were installed for each country to facilitate the organization and distribution of the chemistry PT scheme, to help promote the PT schemes and to reduce shipment costs. In the meantime the microbiology PT needs to be promoted and explained also even when shipment of samples is done door to door.

All local coordinators were requested to give a short report of their recent activities. Not all local coordinators have been as active as one could wish for and there is room for improvement. In Namibia the local coordinators were asked to promote the food PT schemes from Tanzania also. Unfortunately again there were hardly any participants from countries outside the EAC suggesting that promoting these PTs was not to much effect. The question of credibility of the PT schemes was raised. There is no need for the PT provider to be accredited in order to be acceptable to accreditation bodies. As SANAS does most of the accreditation and they accept this PT scheme Oswald will try to contact AFRAC/SADCAS to have them disseminate the information that this PT scheme is recognised.

- **Angola** (Lopez Ferreira Baptista): There are some companies and some governmental laboratories that do water analysis. They have been contacted.
- **Botswana** (Teddy Ditsabatho): There are just 5 laboratories and 2 are governmental institutions. Personal contact was used. Mines are not interested but informed.
- **Burundi** (Leandre Budigiye): For Burundi it was the second time to participate in the workshop. Leandre was appointed last December. Burundi has a water bottling company and private testing services. There are no participants from governmental

labs so far. This year there were three participants. Other laboratories had low interest due to cost, not knowing immediate returns. Samples of the PT were received well. Raising awareness was done by emphasizing the need for water testing. Roughly 1700 water sources exist in Burundi. Routine analysis parameters as total plate count, total feacal coliforms pH, smell, colour, on some samples full chemical analysis. So far there is no accreditation in Burundi. Maybe setting up a national laboratory association might be a good idea.

- **DRC** (Jean-Paul Munongo): 6 laboratories participated in for chemistry 1 for microbiology. Next year there will be 3 more.
- **Ethiopia** (Abdi Duga Jebessa): Ethiopia has epproyimately 90 laboratories. Promotion was done through laboratory association. Most labs do not understand use of PT. Private labs raise question of credibility of PT. PT providers are not accredited.
- **Ghana** (Regina Vowotor): peculiar situation - government labs and commercial labs (medical) do not want to participate. Governmental labs thrive to become accredited. Brochures were sent but only one micro lab participated. Other westafrican countries have a workshop. Motivation for Participation could be striving for accreditation. Workshop used for raising awareness.
- **Kenya** (Jacqueline Kangiri, Timothy Kiarie): 8 participants for chemistry only one for micro: usually one workshop per year is held that is also used for promotion of the scheme. National accreditation service will promote it. Accreditation is pushed. The laboratories management thinks that analysis is so easy anybody can do it they do not see the need for PT schemes.
- **Lesotho** (Mapaseka Makhaba): email communication was difficult. A lab association is to be formed which would be a good platform for promotion of the scheme. There is only one water lab.
- **Madagaskar** (Yves Mong): there are mainly 4 laboratories working in the water area. There is a platform to support the implementation of water and sanitation policy but not very active. There is a microbiology laboratory that wants to participate next year. They still use the leaflet to promote the scheme. Accreditation is the goal.
- **Malawi** (Steve Afuleni): samples were received without problems. 3 labs participated in chemsitry 1 lab in microbiology. Most laboratories do not understand what PTs are about. A national workshop was held and a national laboratory association is to be formed. Hopefully this will help raise awareness.
- **Mauritius** (Shabbir Ghoorun): 6 laboratories are constantly participating 4 are accredited. There are two water companies. Promotion of the PTs is through personal visit and giving all information about the schemes. Samples were received in good condition after only two days.
- **Namibia** (Merylinda Conradie): Raising awareness is tried to achieve through brochures. Laboratories use south african national laboratory association. There is no national laboratory association but a national standards institute that is quite new.
- **Seychelles** (Vivian Radegonde): On Seychelles there is only SBS for the chemistry PT. Samples were received in good condition. Only very few water testing

laboratories in Seychelles.

- **South Africa** (Mare Linsky): She contacted Randwater to get on board as an expert laboratory for the microbiology PT.
- **Tanzania** (Kezia Mbwambo): Promotion was done through TBS seminars and workshops as well as brochures. There are some accredited laboratories. There was a communication problem with the chemistry PT. Potentially 40 laboratories could participate. Next year a seminar for urban watersuppliers is scheduled. There will be a task force for quality assurance (accreditation).
- **Uganda** (Aziz Mukota): There have beeb 5 participants for the last 2-3 years. No problems with distribution of the samples were encountered. Phone contacts to all participants made the distribution really fast. A national workshop was used to promote the PT schemes. *Challenges*: few labs test water. Most use testkits and are not very confident to participate in the PT. Another challenge is the lack of equipment. Element of cost: although the PT scheme is very cheap really compared to other PT schemes (FAPAS etc.) still for some it is too high. Some other parameters requested pH pestizides.
- **Zambia** (Margaret Mashamo): There are only two labs participating. Laboratories have problems understanding what PTs are about. Accreditation is promoted and maybe this will raise more interest. In Zambia a national laboratory association is to be formed (UNIDO project).
- **Zimbabwe** (Penia Mubika): Promotion of the scheme through national laboratory association as well as on the world standards day/world sanitation day. Still not much improvement in terms of participation. There were no problems with sample distribution. Economy improves in the country and laboratories seem to be improving on capacity and equipment.

Reports on training activities following the training of trainiers (ToT)

Reports on training activities following the training of trainers (ToT) showed that in several countries (Tanzania, Uganda, Seychelles and Kenya) workshops were conducted with or without funding by PTB. In Mauritius a planned workshop was cancelled due to lack of participants. In some other countries workshops are planned for 2012. The details are given in the report of the chemistry workshop.

Training – Use of international standards

All participants of the microbiology PT had been asked to give detailed information on the methods used for analysis of the PT samples with their results. Sometimes ISO standards were cited but the methods described did not match the ISO standard(s).

If changes are made in medium used e.g. due to availability problems or other things changed the method stated can only be "modeled after ISO…" or a similar description. The method has then to be validated at laboratory level.

Microbiological analysis of water samples is greatly operationally defined. E.g. methods for detection and enumeration of total coliforms use anything from lactose fermentation (gas and acid production from lactose) to enzyme activity (ß-galactosidase) to describe

this group. It is not surprising that this leads to a very different set of species detected by various methods. The use of many different methods prevents comparison of the results between laboratories. The SADCWaterLab Association has published a recommendation of what methods seem to be suitable for the analysis of potable water in the SADC and EAC region on the SADCMET website. It is also available through the PT provider UNBS.

Due to the mentioned discrepancies between stated and used method the first part of the training focused on the rules that apply to using international standards. ISO 9308-1 (Enumeration of E. coli and Coliform bacteria by a membrane filtration method) was used to analyze in general the essential contents of a standard. If any changes to the critical parts are made like using a different type of medium or different incubation times or temperatures it cannot be considered the application of the standard method. Any such change needs to be carefully validated and the outcome can be a house method called “modelled after” ISO ….. In case the standard is applied properly it is assumed to be validated and the laboratory has only to conduct a secondary validation confirming the method is under control and performing as expected.

For analysis of microbiological parameters in drinking water there are quite a few standard methods published by the ISO Technical Committee 147 “Water Quality” subcommittee 4 “Microbiological methods”. Part of the list can be found in annex 2 “Training microbiology” and up to date information on the methods is always available on the ISO homepage (www.iso.org).

One of the most important things to consider before using a method is to make sure it is fit for purpose. It is essential to know the exact purpose of the analysis. In order to be able to choose a suitable method the laboratory needs to know the type or source of the water sample as well as what limit values should the results be compared to. These limit values are often derived from state laws or regulations.

Training – Membrane filtration methods QC and troubleshooting

During the workshop in Namibia in 2010 a working group of the SADCWaterLab Association was installed to write a recommendation on what methods are considered suitable for drinking water analysis. The membrane filtration method ISO 9308-1 for enumeration of E. coli and coliform bacteria was recommended and a large number of laboratories stated to have used this method for analysis of the PT sample for E. coli. During the Mauritius workshop all steps of membrane filtration methods were discussed in small working groups in detail using ISO 9308-1 as an example. As an outcome the following checklist was developed to help with quality control and troubleshooting within the microbiological laboratory.

What factors influence the quality of the results in a membrane filtration method?

- Cooling of the samples, packaging
- samples not warm to the touch
- suitable containers; integrity of the packages (in case of bottled water)
- check if the right quantity is delivered for the parameters to be analized
- check label: source of sample sampling date; parameters needed; type of water;

preservation (chlorinated water)

- let samples reach room temperature
- check measurment of 100 ml in funnel
- check proper function of medium by using positive and negative controls
- quantitative control for quantitative measurements
- to detect major inhibitory effects of bad membrane/medium interaction target and non-target organisms can be streaked across a plate with membrane placed on agar used
- sterilisation of the filtration apparatus (autoclaving times and temperatures; flaming procedures)
- running blanks with all steps of the methods included or only parts of the methods
- check the within staff repeatability and the between staff repeatability of counting
- sterile forceps , transfer of the membranes
- incubators temperatures independend Thermometer in the incubator reading 1-3 times a day recording it
- plot temperatures in order to easier detect outliers
- humidity of the incubator (drying of the plates) water

For the slides of the presentation of the training sessions see annex 2.

Report of the PT provider

Jacqueline Kwesiga from Uganda Bureau of Standards (UNBS) described the trial runs with Randwater in April of 2011 that were conducted with good outcome. DHL was used as a courier as previously. Unfortunately the shipment of the PT samples had to be postponed twice due to DHL not providing the ordered packaging material in time and then because of quality control failure of the PT preparation. Finally the samples were shipped 4 weeks later then originally planned. Compared to previous rounds the number of participants increased to 40. This is a good development.

The slides of the presentation are given in annex 3.

Evaluation of the PT

General aspects:

A major improvement has been achieved by using repeat analysis of the PT samples by expert laboratories combined with quality control data of the PT provider UNBS to calculate target values. At least in the PT trials and also for one of the samples (total plate counts) this worked well.

Analysis of the reported data revealed that only 10 of the samples were delivered within the optimal 2 day period and 13 showed temperatures in the desired range of below 10°C. Another 6 samples arrived at temperatures below 15°C. In order to further improve the PT scheme packaging and logistics should be optimized before the next PT round. In order to find out if freeze dried material might be more suitable for this regional PT with rather large distances to cover into more remote areas of some African

countries there should be additional freeze dried material alongside the liquid samples. This will be without additional costs to the participants.

Again not all laboratories managed to start analysis of the PT samples at the day of reception although this is crucial as the samples contain live organisms and therefore have limited stability.

As had been decided during the previous workshop in Namibia Randwater had been approached and thankfully agreed to act as an expert laboratory as well as the laboratory of the scientific consultant NLGA (Germany).

E. coli / coliform bacteria: evaluation and assessment

The sample for analysis of E. coli and coliform bacteria contained E. coli in a concentration of approximately 100 - 150 CFU/100 ml. Unfortunately the quality control data of UNBS and the expert laboratories did not match as closely as expected. Some samples showed growth of unexpected types of bacteria so a contamination during the bottling process might have occurred. This sample was not evaluated by statistical means. *This kind of sample quality problems were encountered for the first time during the 4 PT rounds. Extra measures will be taken to prevent that from happening again.*

Most participants reported some kind of method with a rather short description of their proceedings. It was quite obvious that at least some of the described proceedings did not match the cited standard e.g. a medium was used that is not mentioned in the standard. Therefore the standard has not been applied properly.

Total plate counts: evaluation - assessment

Quality control data and results of the expert laboratories for the total plate count samples were in good agreement. Therefore an assigned value of 64 CFU/ml was calculated and used for conducting a statistical analysis of the participants results.

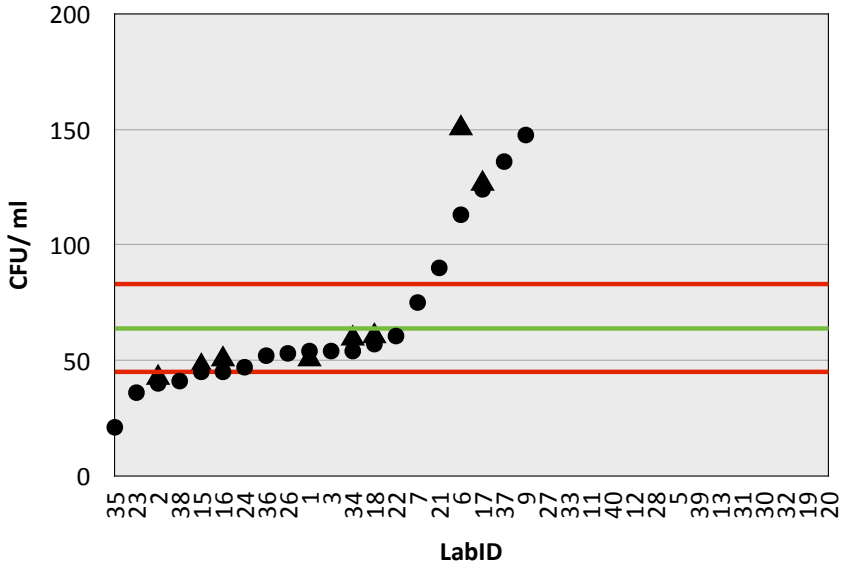

Figure 1: Total plate counts - participants results sorted; red lines: z-score -2 and +2, green line: Alg A mean as assigned value calculated from results of expert laboratories and UNBS quality control data; dots: results for 36°C, triangles: results for 22°C

Out of 35 results reported for total plate counts at 36°C 11 had Z-scores in the range of -2 to +2 (45 – 82 CFU/ml) and can be considered excellent or acceptable. Four additional results fall into the class from -3 to +3 and can be considered questionable.

Almost half of the result (n=15) are at least one or more log scales too high. Some of these might be due to growth of the bacteria during transport. On the other hand there are no nutrient in the transport medium to support such a growth and therefore there might be problems with the reporting of the results in terms of calculating the numbers to the correct volume. Other reasons for these very high results might be difficulties with counting only colonies or contamination of pipettes or plates.

The strain used in the PT grows at lower temperatures e.g. 22°C as well as at 36°C. Therefore the assigned value was used for calculating the z-scores for the lower temperature results as well. Out of 14 results 5 were has z-scores between -2 and +2 an another one was between -3 and +3.

For all laboratories that reported results for two temperatures the two results were close enough to be at least consistent with one exception were there was a tenfold higher number for the lower temperature.

The details of the evaluation are given in annex 4.

Evaluation of the workshop by participants

An evaluation questionnaire was distributed on the last day of the workshop and all participants handed back their answers. The summary is given below.

How do you judge:	**Very good 1**	**good 2**	**fair 3**	**poor 4**	**very poor 5**	**Mean**
The venue of the workshop	10	7	0	0	0	1.4
The hotel (accomodation)	9	6	1	0	0	1.5
How do you judge the different parts of the workshop?	**Very useful**				**not useful**	
Report of the PT provider	4	7	4	2	0	2.2
Evaluation of the PT	6	9	3	0	0	1.8
Training on membrane filtration methods	4	10	3	0	0	1.9
Training on use of ISO methods	3	9	5	0	0	2.1
Intralaboratory quality control	1	9	5	0	0	2.3
SADCWaterlab general assembly	3	8	4	2	0	2.3

Did the workshop fulfill your expectations? Yes/No/Partially If no or partially please explain. Answers: Yes 12 No: 4 Partially: 1

Explanations:

- Inconclusive microbiology results, sample integrity questionable
- It did not fulfill the expectations because the PT provider was not able to provide participants with a report to show whether they were competent or not.
- more detail in quality assurance required
- the time was not enough for more ...and the...would helpt the participant

What were the most important topics to you? (No of participants naming the topic)

Training on ISO methods and how to use them (9)

Training on membrane filtration methods (7)

Evaluation of PT results (5)

Intralaboratory quality control (4)

Report of the PT provider

Preparation and dispatch of PT samples problems encountered personal experience

Internal audits and QMS

Importance of a functional quality management system

Quality control in microbiology labs

PT evaluation, z-scores, robust statistics

What benefits died you draw from the workshop?

- Microbiology method validation (6)
- training on uncertainty of measurement (4)
- QC and QA in microbiological testing laboratories (with specific examples) (3)
- Networking (2)
- the importance of choosing methods that are suitable for the intended purpose.
- The importance of having a management system that will ensure the quality of the results
- The importance of having QA/QC charts plotted on graphs
- The discussions enlightened where as a laboratory mistakes are made and gives a chance to learn and correct the mistakes
- Technique, sharing of experiences and general discussions
- information required for choosing an applicable method
- contents of the report
- interaction with other labs and discussions of views challenges that could be faced when carrying out the methods
- The emphasism on how important it is to continuously attend training to keep up to date with not only new methods but for refreshing oneself
- learning about new techniques
- I have met d/t professionals from different countries. I had the chance to talk with laboratory experts on issues which I had a question
- Training on use of ISO methods and membrane filtration were very essential as some points are not clearly stipulated on the methods but the training made them clear
- Interaction with other laboratories provides me with possibilty of networking and exchange on technical information
- better understanding on the membrane filtration method in the analytics of water
- Training on use of ISO methods (especially 9308-1)

• training on use of ... statistics
• auto evaluation
• familiarity with ISO methods for water microbiolg analysis
• training on self evaluation
• an eye-opener on how PTB PT provider function
• how to evaluate our PT results
• how to better use the ISO methods
• know what is happening in the other participants labs
• better understanding of ISO 9308-1
• how to improve the intralaboratory quality control in the laboratory
• sharing experience with other laboratories on how they operate
• **What topics would you suggest for further training**
• Guidance on equipment calibration requirements
• Provide tools (programs) for statistical evaluation
• training on ISO 17025
• requirements for accreditation
• QC and evaluation of competence of laboratories
• trouble shooting in microbiology labs
• microbiology quality assurance
• other tests for analysis of water
• root cause analysis
• interlab comparisons
• Guidance on staff competency evaluation

Participants list - 4th PT evaluation workshop, Mauritius, November 2011

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Participants list - 4th PT evaluation workshop, Mauritius, November 2011

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SADCMET Water PT
Evaluation Workshop
Microbiology Proficiency Testing

- Training -

International Standards

- what´s out there
- how does it get there
- what they can do for you
- how to use them

- what they **cannot** do for you

TC 147 - Water quality

Items to be displayed:

- [ ] Published standards
- [ ] Withdrawn standards
- [x] Standards under development
- [ ] Projects deleted (last 12 months)

Subcommittees

Subcommittee	Subcommittee Title
TC 147/SC 1	Terminology
TC 147/SC 2	Physical, chemical and biochemical methods
TC 147/SC 3	Radiological methods
TC 147/SC 4	Microbiological methods
TC 147/SC 5	Biological methods
TC 147/SC 6	Sampling (general methods)

TC 147/SC 4 - Microbiological methods

Items to be displayed:

- [x] Published standards
- [ ] Withdrawn standards
- [x] Standards under development
- [ ] Projects deleted (last 12 months)

Standards and projects under the direct responsibility of TC 147/SC 4 Secretariat

Standard and/or project	Stage	
ISO 6222:1999 Water quality -- Enumeration of culturable micro-organisms -- Colony count by inoculation in a nutrient agar culture medium	90.93	07
ISO 6461-1:1986 Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 1: Method by enrichment in a liquid medium	90.93	07
ISO 6461-2:1986 Water quality -- Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) -- Part 2: Method by membrane filtration	90.93	07
ISO 7704:1985 Water quality -- Evaluation of membrane filters used for microbiological analyses	90.93	07
ISO 7899-1:1998 Water quality -- Detection and enumeration of intestinal enterococci -- Part 1: Miniaturized method (Most Probable Number) for surface and waste water	90.93	07
ISO 7899-1:1998/Cor 1:2000	60.60	07
ISO 7899-2:2000 Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method	90.93	07

ISO 8199:2005 Water quality -- General guidance on the enumeration of micro-organisms by culture	90.93	07.100.20
ISO/CD 9308-1 Water quality -- Enumeration of Escherichia coli and coliform bacteria -- Part 1: Membrane filtration method for waters with low bacterial background flora	30.60	07.100.20
ISO 9308-1:2000 Water quality -- Detection and enumeration of Escherichia coli and coliform bacteria -- Part 1: Membrane filtration method	90.92	07.100.20
ISO 9308-1:2000/Cor 1:2007	60.60	07.100.20
ISO 9308-2:1990 Water quality -- Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli -- Part 2: Multiple tube (most probable number) method	90.92	07.100.20
ISO/DIS 9308-2 Water quality -- Enumeration of Escherichia coli and coliform bacteria -- Part 2: Most probable number method	40.99	07.100.20
ISO 9308-3:1998 Water quality -- Detection and enumeration of Escherichia coli and coliform bacteria -- Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of E. coli in surface and waste water	90.93	07.100.20
ISO 9308-3:1998/Cor 1:2000	60.60	07.100.20
ISO 9998:1991 Water quality -- Practices for evaluating and controlling microbiological colony count media used in water quality tests	90.93	07.100.20
ISO 10705-1:1995 Water quality -- Detection and enumeration of bacteriophages -- Part 1: Enumeration of F-specific RNA bacteriophages	90.20	07.100.20
ISO 10705-2:2000 Water quality -- Detection and enumeration of bacteriophages -- Part 2: Enumeration of somatic coliphages	90.93	07.100.20
ISO 10705-3:2003 Water quality -- Detection and enumeration of bacteriophages -- Part 3: Validation of methods for concentration of bacteriophages from water	90.93	07.100.20

International Standards

- ISO International Standardization Organization
- Technical committees
- Member Bodies: national standardization bodies
- written and discussed by experts
- well known and used method
- best available „compromise“

Stages in the development process of an ISO standard[12][13][14][17][18]

Stage code	Stage	Associated document name	Abbreviations
00	Preliminary stage	Preliminary work item	PWI
10	Proposal stage	New work item proposal	NP or NWIP, NP Amd/TR/TS/IWA
20	Preparatory stage	Working draft(s)	AWI, AWI Amd/TR/TS, WD, WD Amd/TR/TS
30	Committee stage	Committee draft(s)	CD, CD Amd/Cor/TR/TS, PDAmd (PDAM), PDTR
40	Enquiry stage	Enquiry draft	DIS, FCD, FPDAmd, DAmd (DAM), FPDISP, DTR
50	Approval stage	Final draft International Standard	FDIS, FDAmd (FDAM), PRF, PRF Amd/TTA/TR, FDTR
60	Publication stage	International Standard	ISO TR, TS, IWA, Amd, Cor
90	Review stage		ISO TR, TS, IWA, Amd, Cor
95	Withdrawal stage		

ISO 9308-1

- **Working group questions:**

- Are you familiar with the standard at hand?
- How often Do you use it?
- List the most important contents of the standard

Standards contain

- normative information
- informative information
- definitions
- precise technical information
  - instrumentation
  - media composition
  - times
  - temperatures
- format of how to report the results
- most often encountered problems

- What is your purpose?
- Is the standard fit for your purpose?

- To establish a standard method only secondary validation is needed.

Methods PT4

- **Does the description meet ISO 9308-1? If not why?**

1	diluent;1/4 strength Ringers solution; Membrane Filtration Method, using Lactose TTC agar,confirmation tests: Oxidase negative & Indole positive	
2	37°C over night on mLGA	
3	Media violet red bile glucose agare temperature 37+-1°C 24 h	
4	37°C/24h Gelose Tergitol galerie API20E, Gelose trytonee au soya (TSA)	
5	0.47um membrane filter on Tergitol-7-Agar. Incubated at 37degrees for 24 hours. Confirmed using oxidase and indole.	
6	Tergitol7 37°C 21 h Indole and Oxides	
7	Tergitol-7 at 37oC, for24h, Tryptophan broth at 44oC for 24h and Kovacs reagent	
8	100 ml of sample was filtered on a cellulose acetate membrane filter and placed on Lactose TTC agar for 24 hours at 37oC. Typical colonies were picked up to perform oxidase test and also inoculated in tryptophan broth and incubated at 44oC for 24 hours. Indole test were performed on each tube to confirm presence of E. coli.	
10	Membrane filter technique and incorporation on TTC agar. After 24 hours of incubation: - in 37°C for the total coliforms,- in 44°C for the E.coli	
11	37°C, 24h, Tergitol7 Agar confirmed bi Oxides test and growth´Wh on TBX	
12	Lactose TTC with Tergitol-7 (SIGMA-ALDRICH), Incubation temperature 35±2°C for 18-24hrs	

Internal quality control - MF methods

- What factors influence the quality of the result in a membrane filtration method?

QC - Membrane filtration

- cooling of the samples, packaging
- samples not warm to the touch
- suitable containers; integrity of the packages (in case of bottled water)
- check if the right quantity is delivered for the parameters to be analized
- check label: source of sample sampling date; parameters needed; type of water; preservation (chlorinated water)
- let samples reach room temperature
- check measurment of 100 ml in funnel
- check the midium positive control negegative control
- quantitative control for quantitative measurements

QC - Membrane filtration

- membrane check Streaking of organisms
- sterilisation of the filtration apparatus
- running blanks
- check the within staff repeatability and the between staff repeatability of counting
- sterile forceps , transfer of the membranes
- incubators temperatures independend Thermometer in the incubator reading 1-3 times a day recording it
- plot temperatures easier to pick outliers
- humidity of the incubator (drying of the plates) water

Quality control

- membranes material / pore size
- medium (preparation/function)
- volume measurements
- incubation temperatures
- incubation times
- membrane-agar interaction
- sterilisation of the funnel
- transfer of the Membrane
- moisture of the plate

SADCWATER LAB EVALUATION WORKSHOP

Jacqueline Kwesiga

Report of the PT provider

Mauritius – 13th November 2011

Background PT 4 -2011

- During the previous year at the evaluation workshop 2010 agreed to include at least one expert lab in 2011's PT exercise:
- Reasons were primarily:

1. To get consensus assigned values for the parameters analysed in this PT scheme. This was because in the past this had proven to be very difficult to obtain from the participants results.

Background PT 4

- In the first quarter 2011 2 labs did agree to become our expert labs:

1. Rand Water in South Africa.

2. NLGA in Germany.

Background PT4

- To assess whether inclusion of the expert labs in this PT scheme would work as envisaged. A trial run was carried out in early April 2011.

- UNBS sent samples to RandWater and NLGA and also UNBS went on ahead to carry out homogeneity and stability tests.

Background PT4

- The results of this trial were quite encouraging because UNBS, NLGA and Rand Water had values more or less in the same range.

Background PT4

- Hence this gave all the parties concerned greater hope that this could be repeated in August 2011 with even greater success.

PT round 4 - Preparations

- The 1st notification for participants to register was out on 15th February 2011.
- A 2nd and remainder notification was sent out on 7th April 2011.
- Deadline for all these notifications was 1st May 2011.
- A total of 40 labs registered. A significant improvement from last year where 33 labs registered.
- The bacteria were grown and harvested on the 22nd August 2011. Spiking of the bulk liquid PT transport media was on the 26th August 2011 and bottling was carried out on 27th August 2011 after homogenisation.

PT round 4 - Preparations

- One sample (A) was intended for analysis of E. coli and Coliform bacteria the second (B) for heterotrophic (or total) plate counts. Every participating laboratory received one bottle with a portion of each prepared sample.
- The package contained a third bottle (C) with water that served as a temperature control. The temperature in this bottle had to be measured as the package was opened and gave an indication whether the sample temperature was in the desired range of below 10° C.

PT round 4 - Preparations

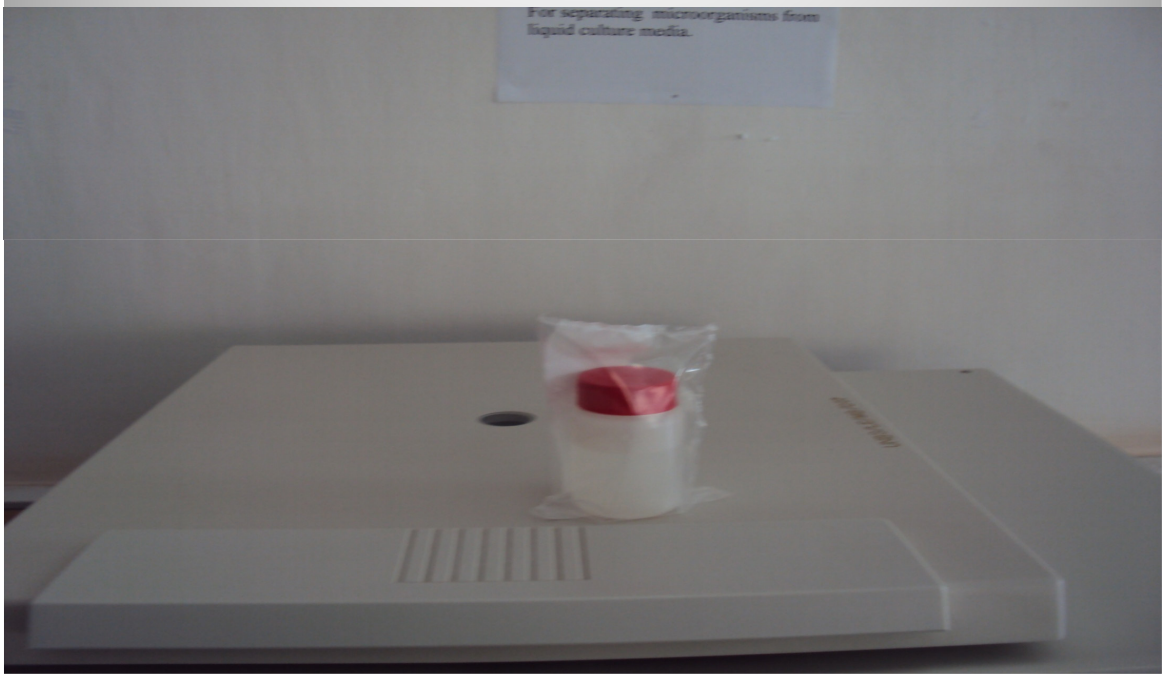
- The PT was distributed on Monday the 29th August 2011 so that participants would be able to receive their packages during the week and analyse them immediately.
- The courier used was DHL.
- A preliminary evaluation report was distributed on 9th November 2011

PT round 4 - packaging

- A uniform styrofoam packaging was used all round. Dimensions of Styrofoam packaging: 33 (l) X 19 (w) x 27 (h) cm
- It contained 4 or 5 pieces of hard shell ice bricks that had been stored in a domestic type freezer at -33^{0}C for 72 hours due to the temperature sensitive nature of the samples
- As agreed previously, the sample bottles A, B and C received by all the laboratories were bagged to minimise on spillages during transportation. Packaging was also done to restrict as much as possible movement within the packages by including in pieces of styrofoam which would restrict movement during the usually rough handling at ports of entry.

PT round 4 - Packaging

PT round 4 - packaging



PT round 4 - packaging

PT round 4 - Packaging

PT round 4 - packaging

PT round 4 packaging

PT round 4 packaging

PT round 4 - packaging

- Also included in the packaging was an excel worksheet for reporting of results that had also been previously emailed to the participants as well as an accompanying letter containing instructions on how to treat the samples.
- Unlike before the results sheets were emailed to the participants because of there past losses during the transit period.

PT round 4 – Logistics / Packaging

- The participants were also emailed there respective shipment way bills so that they would be responsible for tracking and monitoring there packages in the case of any problems for example impounding at customs.

PT Round 4 challenges – methods of analysis used.

- A lot of the labs did not use the recommended methods of analysis as discussed and agreed by the Microbiology WG in Namibia 2010.
- To ensure that all had received these recommended methods of analysis the PT provider emailed these in the 1st and 2nd notification of the Microbiology PT.

PT Round 4 challenges – Methods of analysis used

- Use of the recommended methods of analysis is very important because in Microbiology the method used very much influences the outcome of the analysis because the measurand is often defined by the method.

PT round 4 challenges

- Inadequate temperature control along the transportation chain.
- Of the 35 labs that returned results only 13 received there packages at temperatures below 10^0C – the desired temperature range.
- It was also evident in instances were more than one lab was in the same country but several kilometres apart the labs did not receive there samples at the same temperature.

PT round 4 challenges/ Inadequate temperature control during transit.

- The PT samples are very temperature sensitive. High temperatures – bacteria multiply rapidly
- This problem could be attributed to the poor infrastructural problems found in a lot of places in Africa for example:
- Lack of temperature controlled delivery vans needed for long distance delivery.
- Lack of cold room facilities at all the DHL stations or agents offices in some countries.
- Also DHL is not present in all the smaller towns were some of the labs might be situated and this brings about longer delivery times from for instance from the capital cities to the smaller towns where the labs are situated.

PT round 4 challenges – Long delivery times in some instances.

- Only 10 samples were delivered within the optimum 2 day period. 16 samples were delivered on day 3. This is out of a total of 35 labs which returned results. Ideally all the labs should have received there samples within 2 days. However in some instances the connecting flights to the different parts in Africa are not readily available.

PT round 4 challenges – Power rationing in Kampala, Uganda

- There has been for several months now in Kampala, power rationing from 6 hours – 24 hours every alternate day.
- UNBS testing laboratories have a standby generator which had developed a mechanical fault – not realised early because of weekend.
- Quite possible this affected the refrigerated PT samples particularly sample A for the analysis of Total coliforms /E.coli.

PT round 4 challenges - Packaging

- One participant complained that there was a sample leakage.
- Some measures were taken to prevent leakage of the samples for example bagging of each sample.
- Carrying out elaborate labelling on the commercial invoices.
- However, also should note the samples are handled in a not so gentle manner manner both by courier at the ports – witnessed this in Uganda despite precautionary measures.

PT round 4 challenges - costs

- The cost of the packaging materials keeps on escalating – The cost of the outer insulating packaging alone went up to US $ 41. A lot of other inputs are needed in this scheme for example media, supplements, ice bricks and so on without including the transportation costs.
- Would like to propose that in future participating labs pay US $ 150 as opposed to the US$ 100 that they have been paying till presently.

Thank you and Any contributions as to how this PT scheme can be improved with regard to what has been discussed?

PT round 4 challenges - Methods

- Some participants did not properly use or did not use at all the recommended methods of analysis as had been discussed and presented by the Microbiology Working Group at the evaluation workshop 2010.
- Effort was also made on the part of the PT provider to send out this submission to all the participants when the notifications for the PT was sent out.

SADCMET Water PT
Evaluation Workshop
Microbiology Proficiency Testing
4th Round

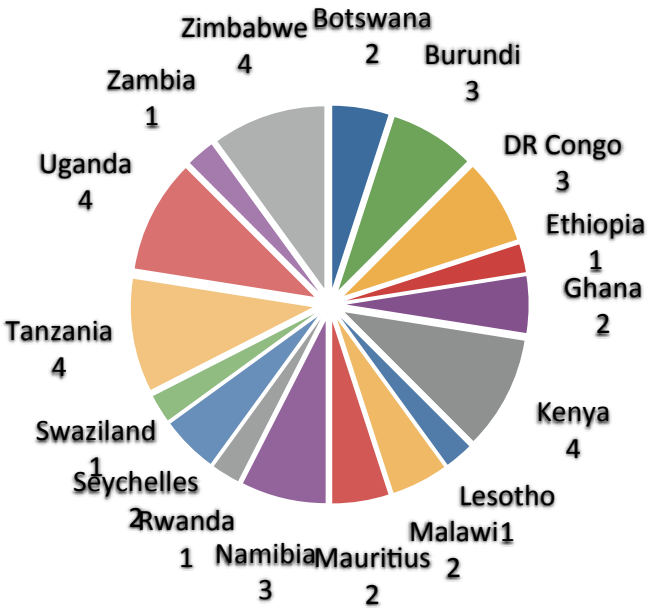
Timeline

- 1st Notification February 2011
- 2nd Notification April 2011
- Registration 01.05.2011
- Shipment (postponed twice) 29.08.2011
- Deadline 12.09.2011
- Preliminary evaluation report November 2011
- Workshop 14.-17.11.2011

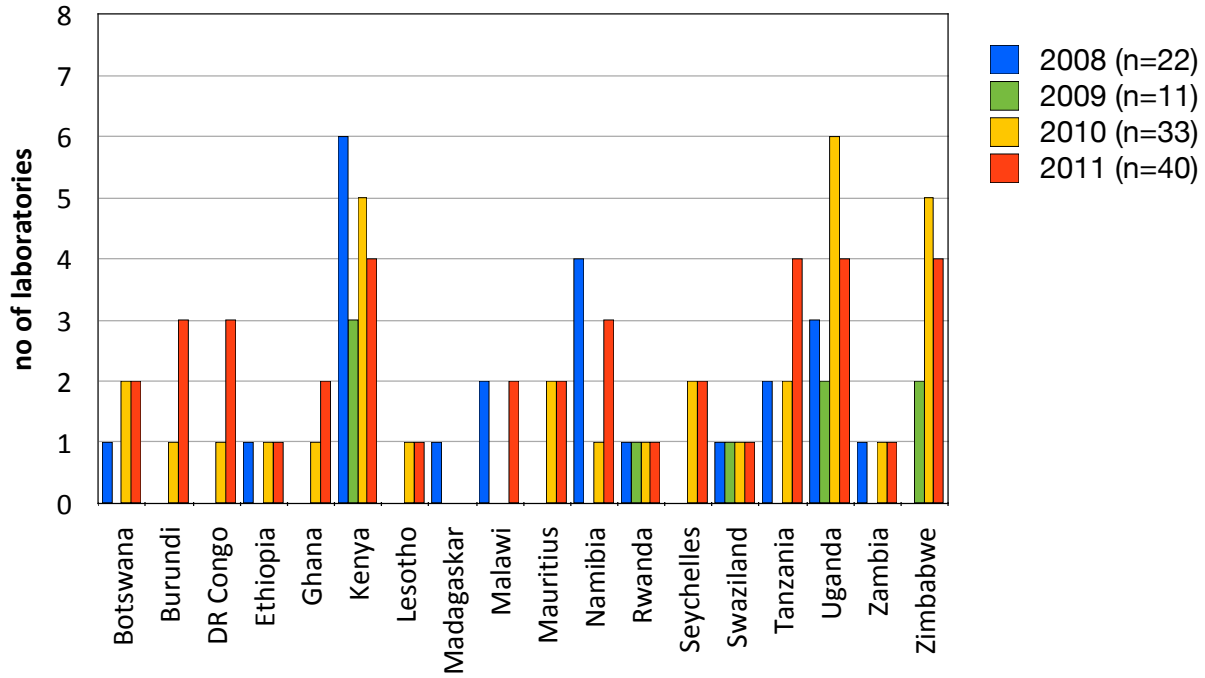
Principle of the PT scheme

- Liquid samples with living organisms
- Samples imitate real water samples
- Handling very much like normal samples
- Limited Stability (7-10 days)
- Samples have to be stored at <10°C
- Imediate analysis necessary

Participation



PT development



General information

- Courier DHL
- Bottle A: 2x concentrate
  E. coli and coliform bacteria
- Bottle B: Total plate counts
- Bottle C: quality control (QC) for determination of temperature upon arrival at the laboratory

Evaluation part 1: Provider

- Performance of the PT provider
  - Communication
  - Preparation of the samples
  - Logistics

Communication

- At the 2010 workshop the next microbiology PT was scheduled for August 2011
- UNBS (J. Kwesiga) gave a first notification as scheduled in February
- 2nd notification in April
- Email Communication: problems?
- Delay 1: Packaging material (DHL) not available
- Delay 2: Preparation problems (power shortage)

Preparation of samples

Quality control

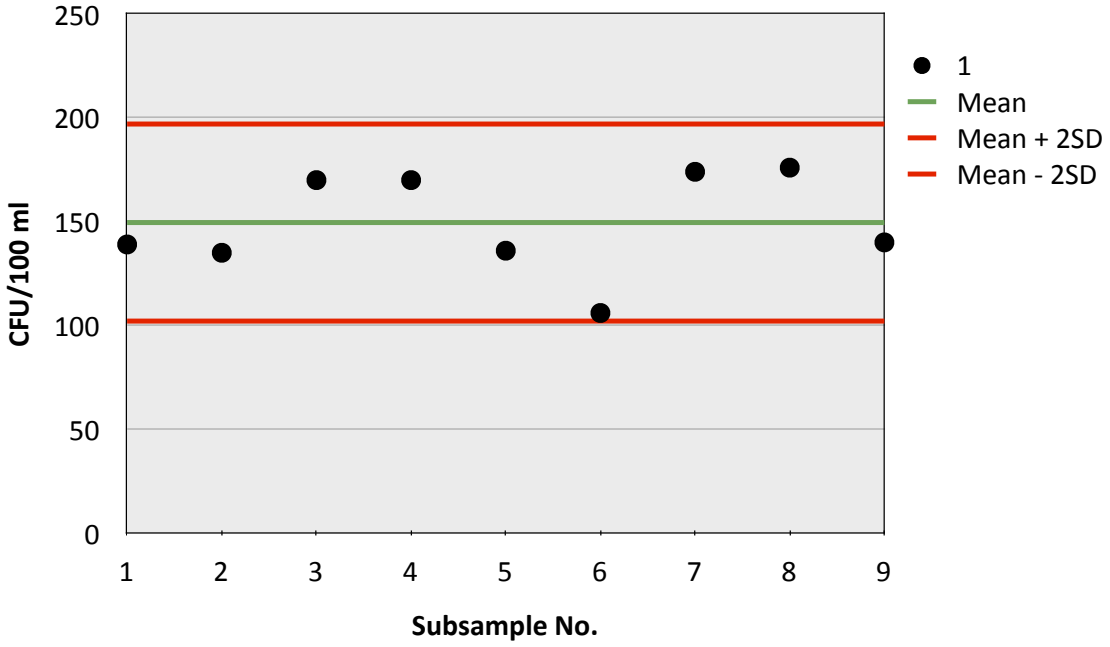
- Homogeneity Testing under repeatability conditions at UNBS:

  E. coli/Colif. day 3 9 bottles Lactose TTC
  TPC day 4 20 bottles 2 replicates

- Stability testing:

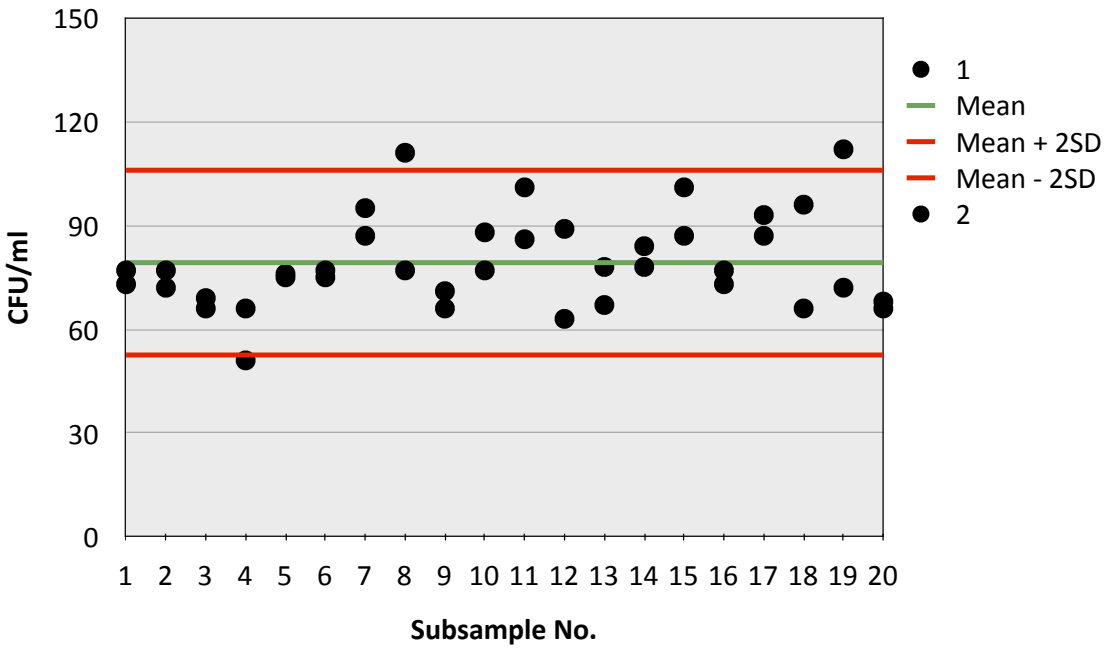
  E. coli/Colif. 1 bottle every (5 d)
  TPC 1 bottle every day 2 replicates (10 d)

Homogeneity E. coli/Colif.



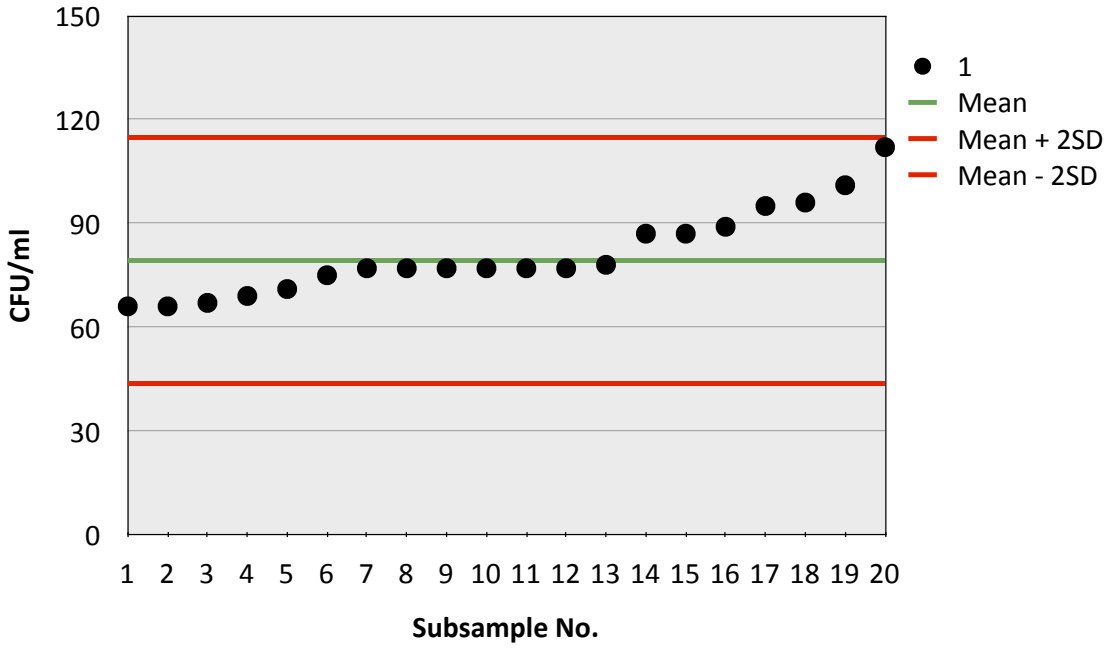
ISO 9308-1 (Membrane filtration, lactose TTC agar) was used for analysis on day 3 after dispatch (01.09.2011), relative standard deviation ~16 %

Homogeneity Total Plate Counts



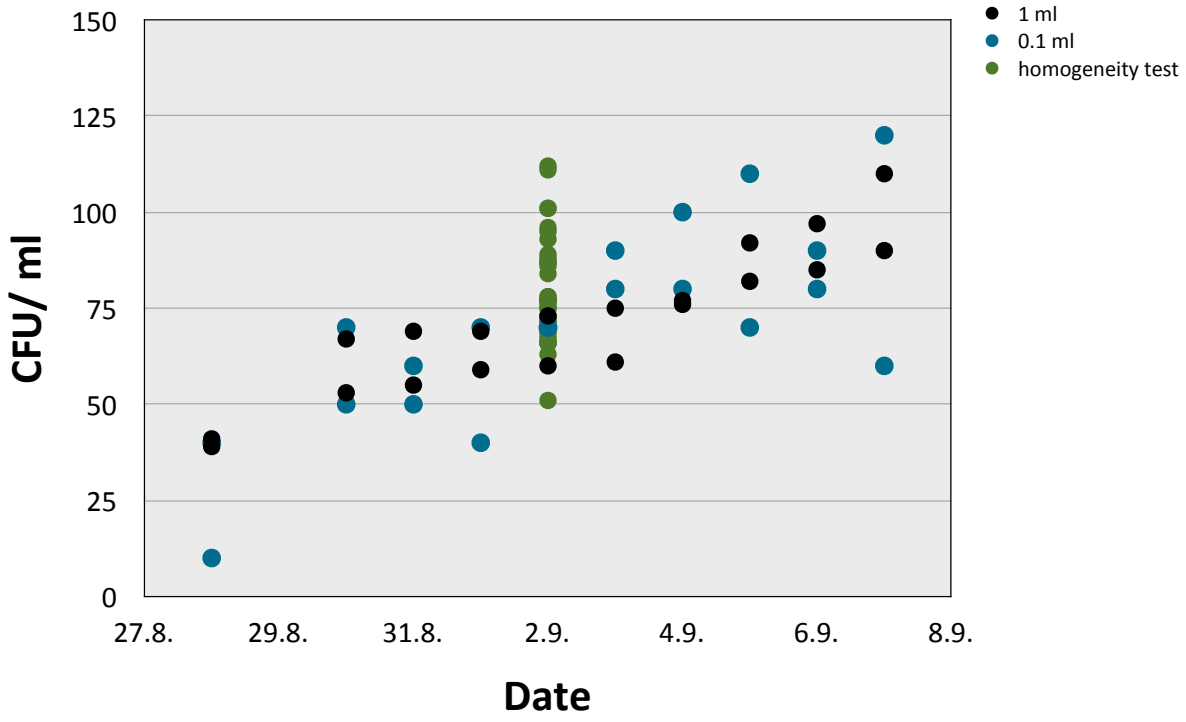
Total plate count method was used with two replicates per bottle (02.09.2011)

Homogeneity Total Plate Counts

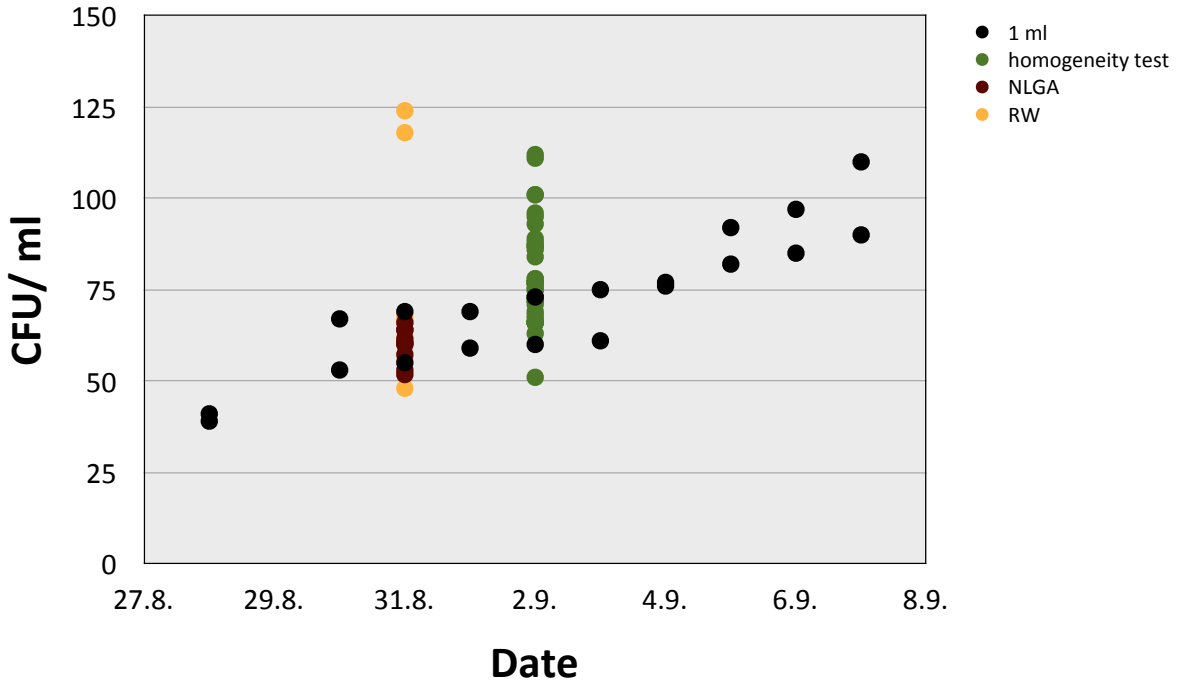


Total plate count method was used with two replicates per bottle (02.09.2011)

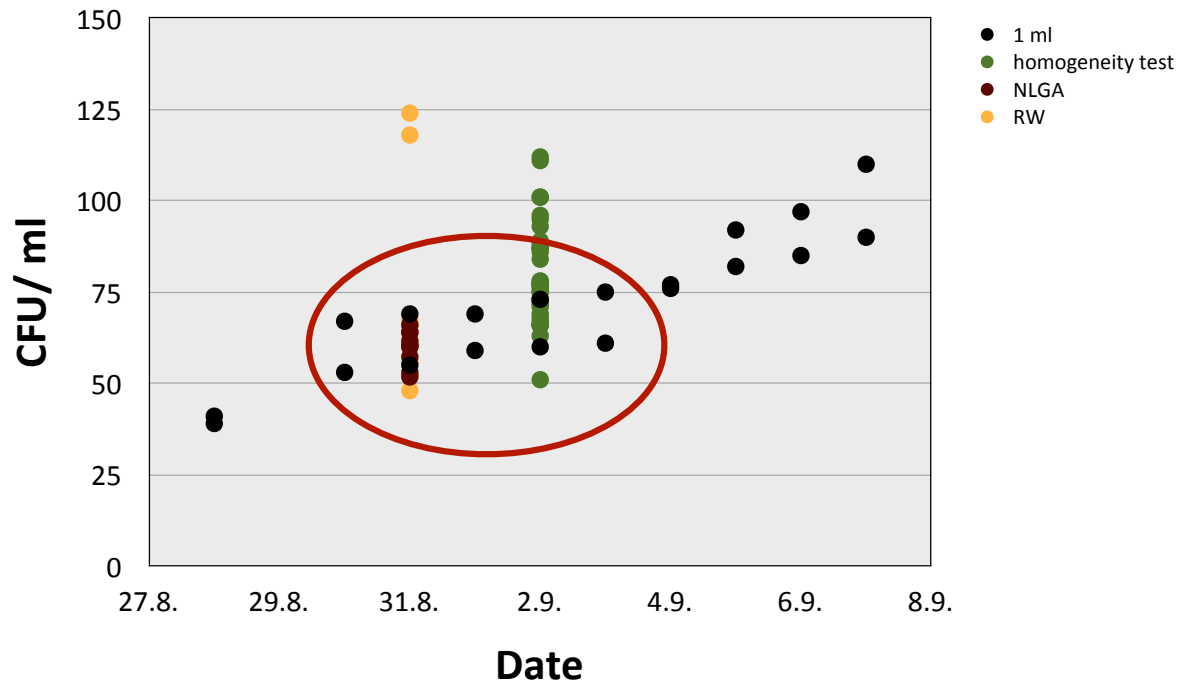
Stability Total Plate Counts



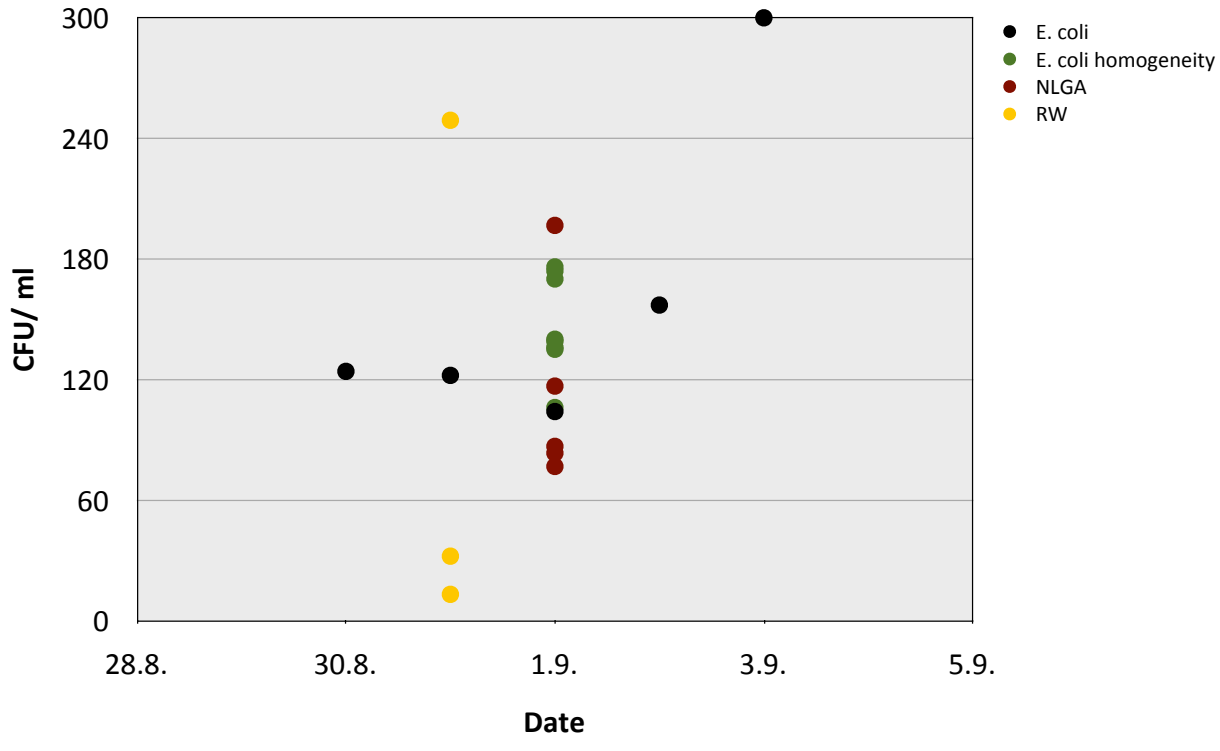
Stability Total Plate Counts



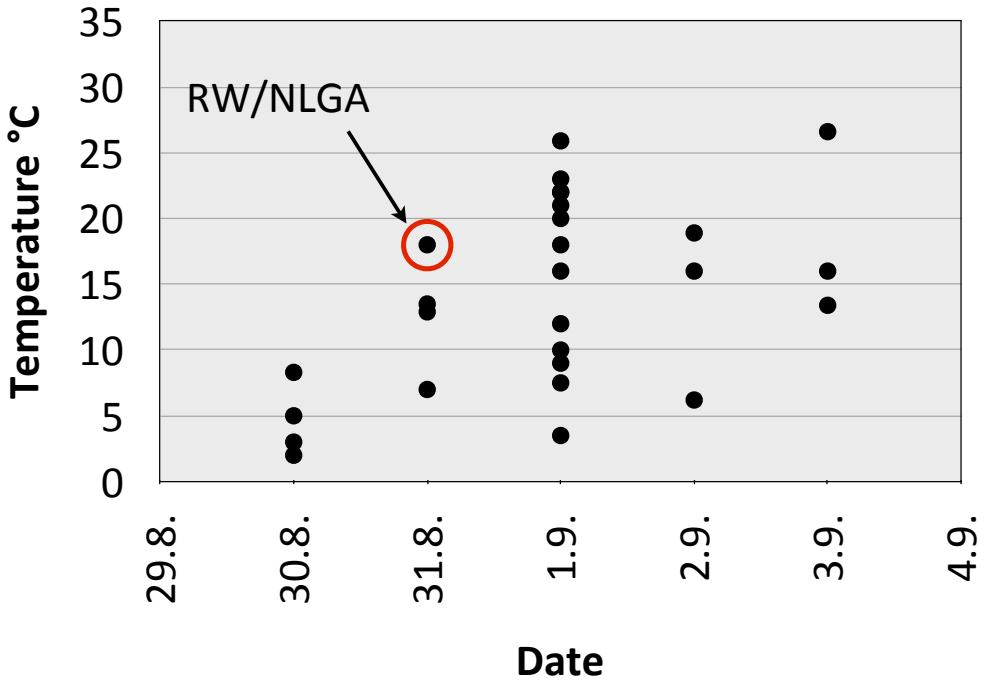
Stability Total Plate Counts



Stability E. coli/Colif.



Logistics - Delivery times and temperatures

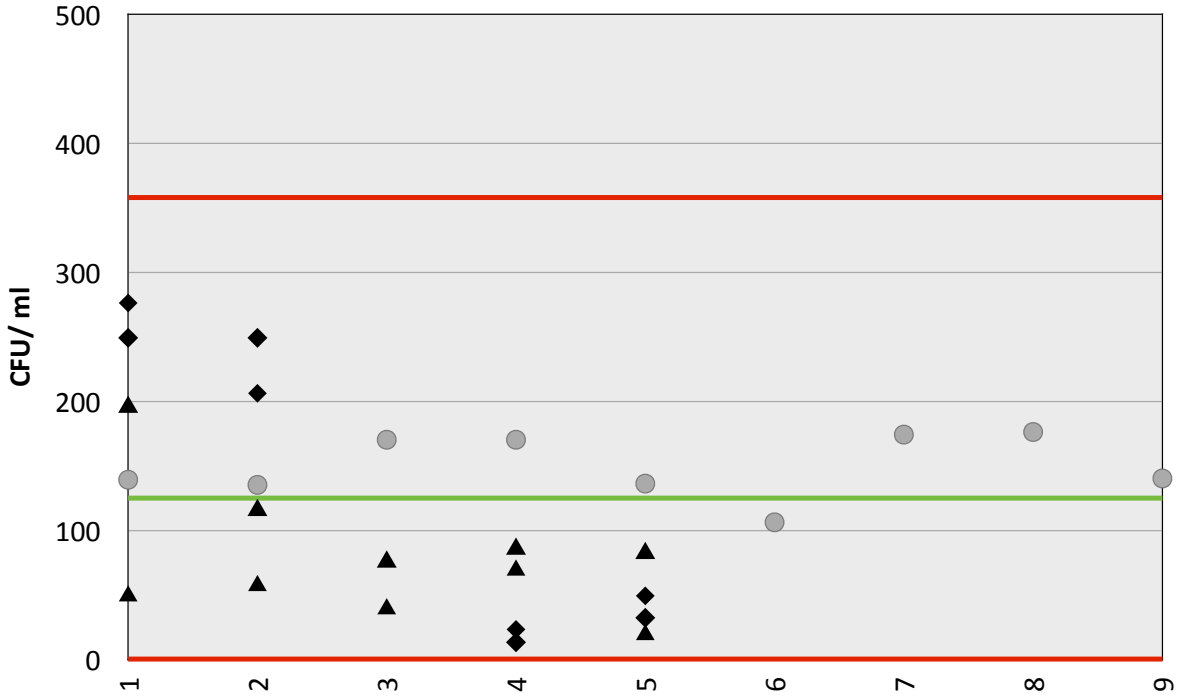


Preparation of samples

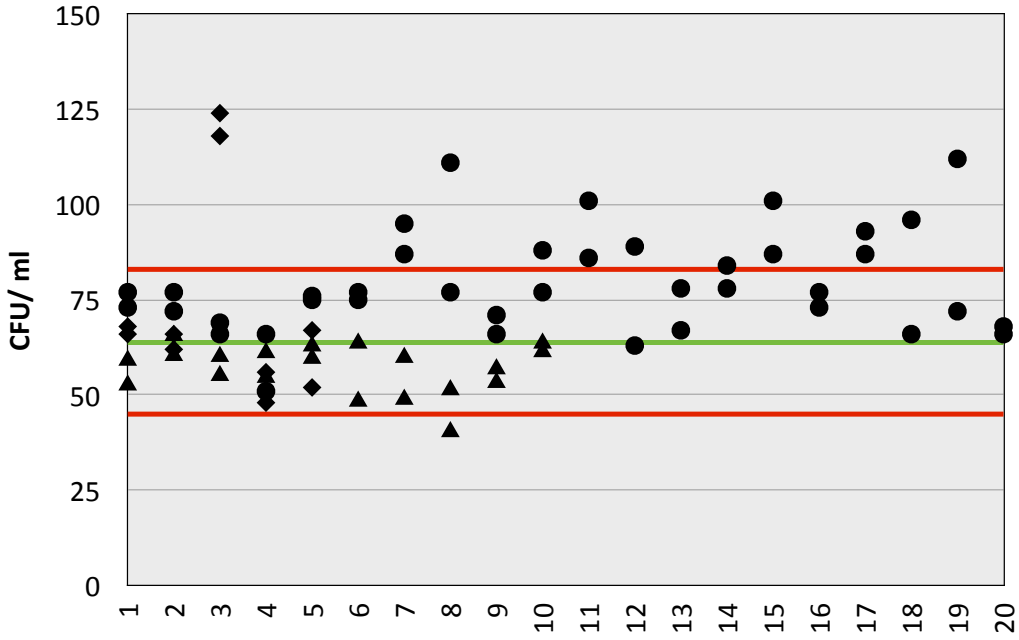
Quality control

- Homogeneity: seemed to be OK in both samples
- Stability: limited as expected but growth started early - room for improvement
- something odd about sample A (E. coli)

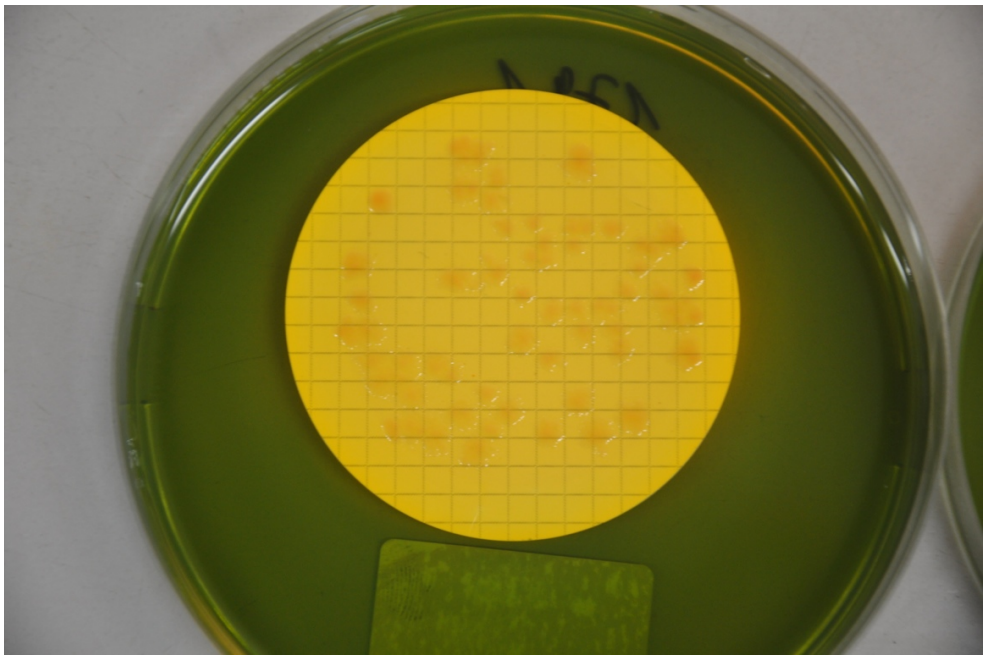
E. coli / Coliform bacteria



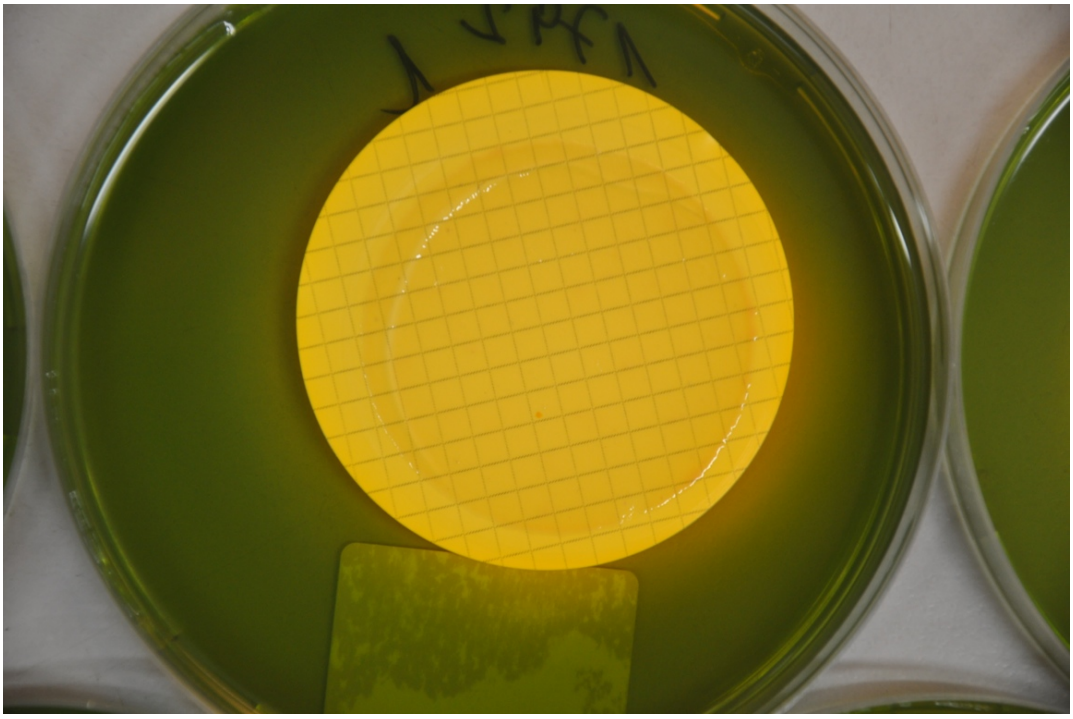
Total plate count (expert labs)



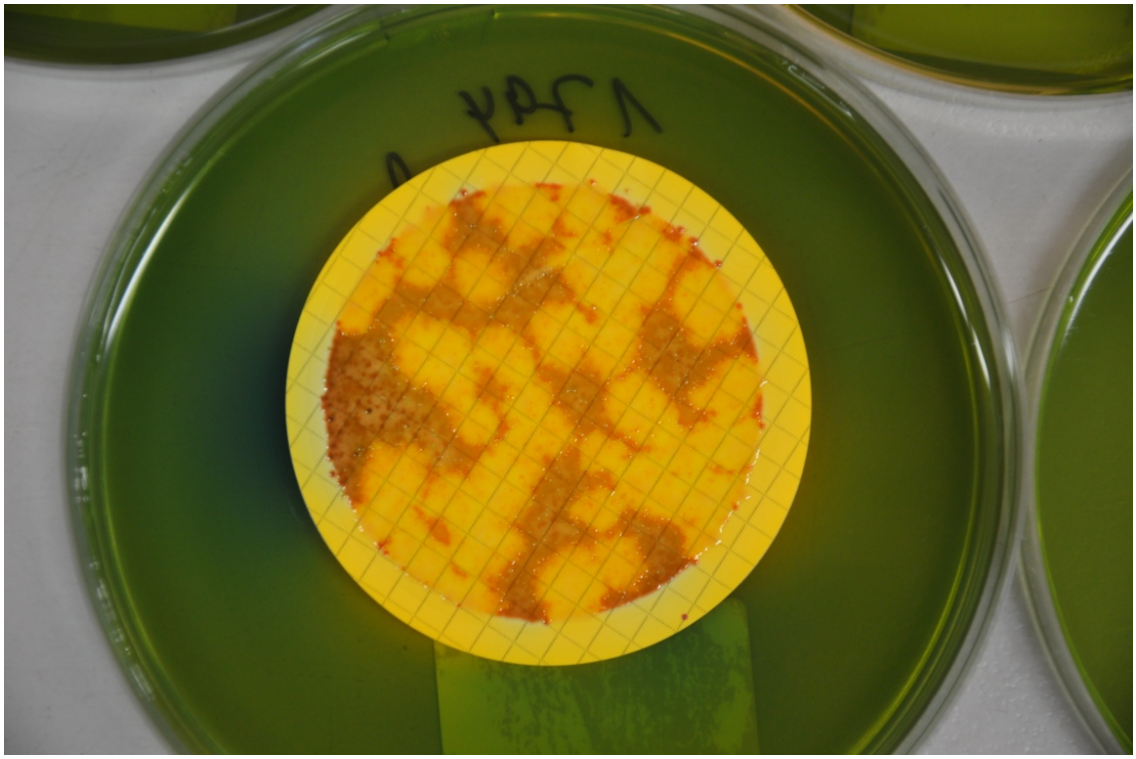
ISO 9308-1



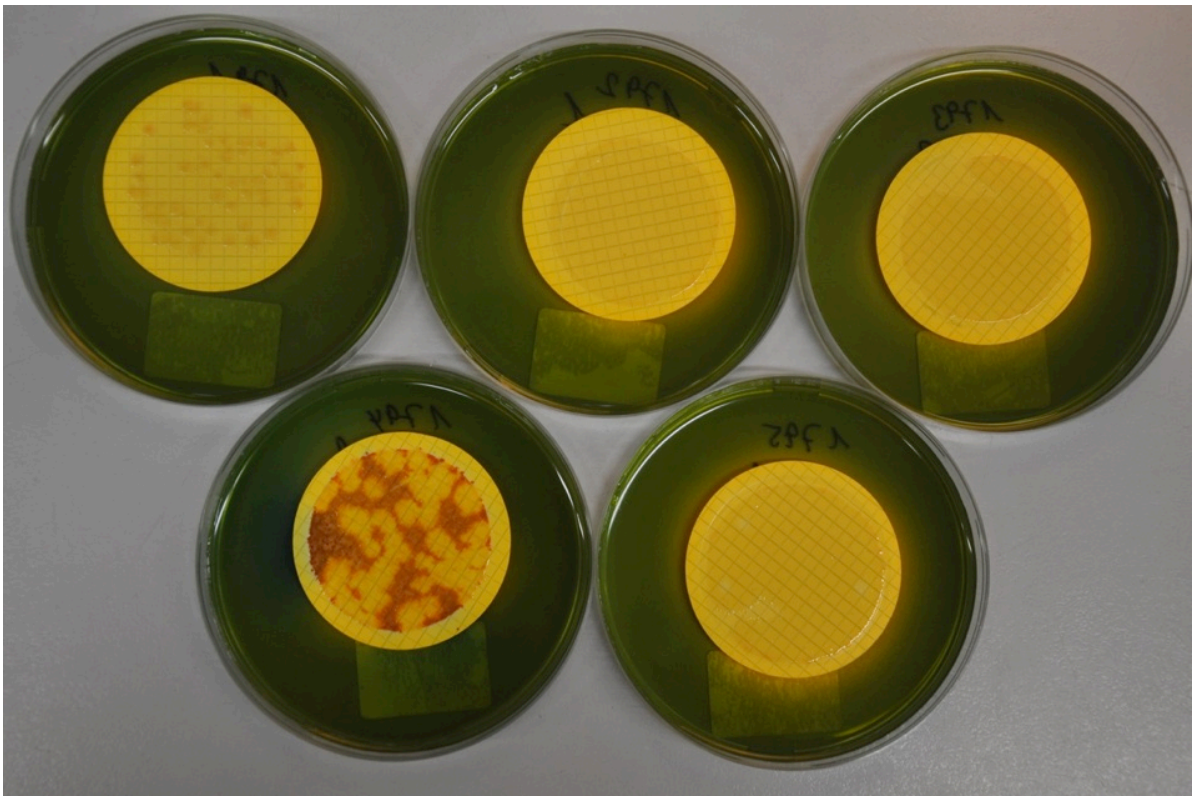
ISO 9308-1



ISO 9308-1



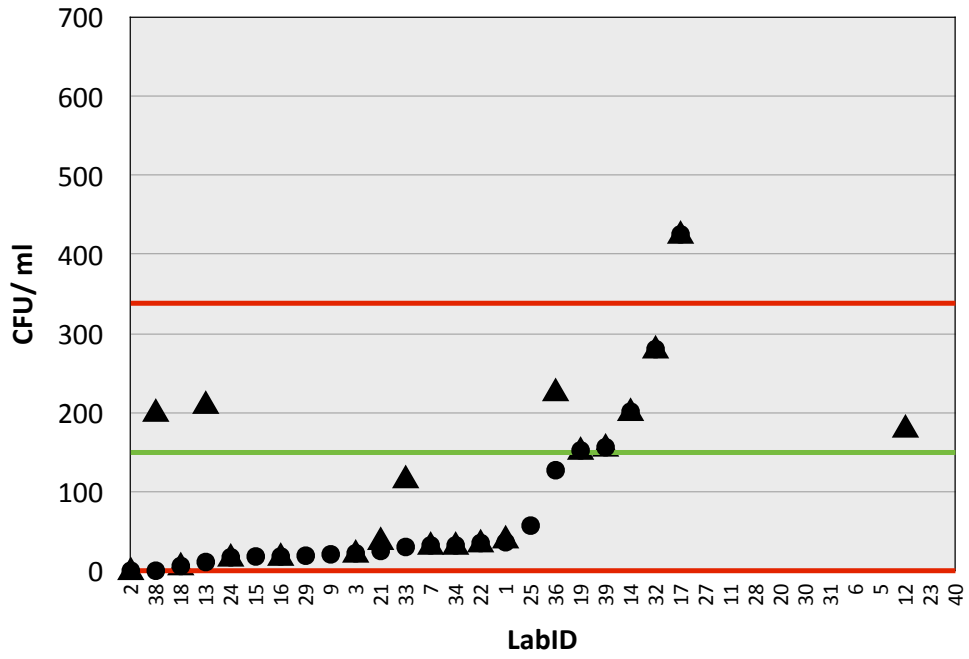
ISO 9308-1



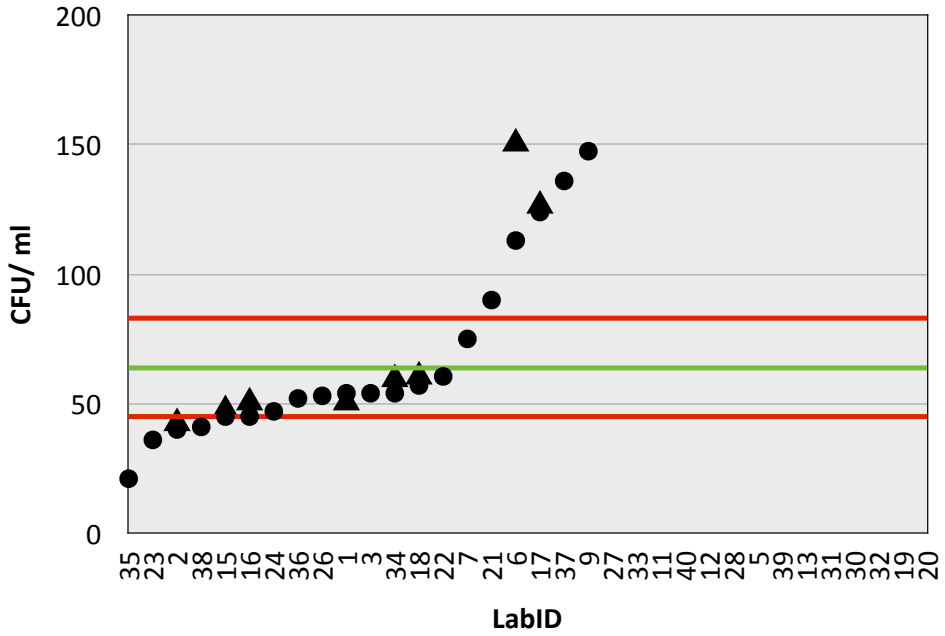
Logistics

- *Delivery: 10 out of 35 samples reached their destination within the optimal 2 day period and 13 (19) samples showed temperatures in the desired range at reception*
- *Although there is no direct correlation between sample temperature at reception and number of bacteria detected. Clearly the transport conditions are a major negative influence on the outcome of the result.*
- *Packaging has to be redesigned*
- *again: Delivery times were not met by DHL*

Results sample A

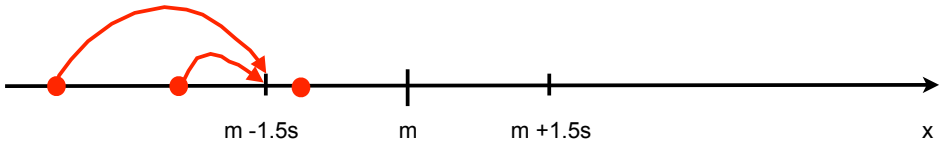


Total plate count



ISO 13528 - AlgA

- Robust method to estimate a mean and standard deviation from participants results
- Arithmetic mean and SD are calculated
  -1.5 s < m < +1.5 s are transformed:
  x ≤ m -1.5 s to m -1.5 s
  x ≥ m +1.5 s to m +1.5 s
- Calculation of Arithmetic mean and SD repeated (iterative calculation possible)



z-score

$$z = (x_i - x^*)/s^*$$

with

x_i: result of participant

x*: AlgA mean from expert laboratories results and QC data UNBS

s*: AlgA standard deviation

used to normalize results and make the outcome comparible and independend of the level of the measurand

LabID	E. coli CFU/ 100 ml	z-score	Coliform	z-score	Temp.	Delivery date	Date of Analysis
2	0	-1,584	0	-1,584	10	01.09.2011	
38	0	-1,584	200	0,537	7	01.09.2011	
18	6	-1,521	6	-1,521	7	31.08.2011	31.08.2011
13	11	-1,468	210	0,643	23	01.09.2011	
24	17	-1,404	17	-1,404	12,9	31.08.2011	
15	18	-1,394			7	31.08.2011	
16	18	-1,394	18	-1,394	9	01.09.2011	
29	19	-1,383			13,4	03.09.2011	
9	20,7	-1,365			-2	31.08.2011	
3	22	-1,351	22	-1,351	2	30.08.2011	
21	25	-1,319	38	-1,181	16	02.09.2011	
33	30	-1,266	116	-0,354	5	31.08.2011	
7	32	-1,245	32	-1,245	16	01.09.2011	
34	32	-1,245	32	-1,245	18	31.08.2011	
22	35	-1,213	35	-1,213	3	30.08.2011	
1	36	-1,203	40	-1,160	3,5	01.09.2011	
25	57	-0,980			22	01.09.2011	
36	127	-0,238	226	0,812	12	01.09.2011	
19	152	0,028	152	0,028	16	03.09.2011	
39	156	0,070	156	0,070	26	05.09.2011	
12	180	0,324	180	0,324	22	01.09.2011	01.09.2011
14	201	0,547	201	0,547	18	01.09.2011	01.09.2011
32	280	1,385	280	1,385	6,2	02.09.2011	
17	425	2,923	425	2,923	23,3	07.09.2011	
27	4600	47,201	4600	47,201	22	01.09.2011	
11	15000	157,498	15000	157,498	25,9	01.09.2011	01.09.2011
28	49000	518,086	70000	740,802	23	01.09.2011	
20	105.000	1111,995	0 other than E. coli		27,5	08.09.2011	
30	24.000.000	254530,951	24.000.000	254530,951	26,6	02.09.2011	
31	>1800		>1800		18,9	02.09.2011	03.09.2011
6	>300		>300		20	01.09.2011	
5	1800+			-1,584	21	01.09.2011	
23	NIL		NIL		8,3	30.08.2011	
40	present		8.300	86,441	21	01.09.2011	
26			not detected		13,5	31.08.2011	
35			14	-1,436	11,1	05.09.2011	
37			28	-1,288	7,5	01.09.2011	

LabID	Temp.	DHL tracking	Delivery date	Date of Analysis
3	2	30.08.2011	30.08.2011	
22	3	30.08.2011	30.08.2011	
33	5	30.08.2011	31.08.2011	
23	8,3	30.08.2011	30.08.2011	
15	7	31.08.2011	31.08.2011	
18	7	31.08.2011	31.08.2011	31.08.2011
24	12,9	31.08.2011	31.08.2011	
26	13,5	31.08.2011	31.08.2011	
34	18	31.08.2011	31.08.2011	
25	22	31.08.2011	01.09.2011	
37	7,5	01.09.2011	01.09.2011	
16	9	01.09.2011	01.09.2011	
2	10	01.09.2011	01.09.2011	
35	11,1	01.09.2011	05.09.2011	
36	12	01.09.2011	01.09.2011	
7	16	01.09.2011	01.09.2011	
14	18	01.09.2011	01.09.2011	01.09.2011
6	20	01.09.2011	01.09.2011	
5	21	01.09.2011	01.09.2011	
40	21	01.09.2011	01.09.2011	
12	22	01.09.2011	01.09.2011	01.09.2011
27	22	01.09.2011	01.09.2011	
13	23	01.09.2011	01.09.2011	
28	23	01.09.2011	01.09.2011	
11	25,9	01.09.2011	01.09.2011	01.09.2011
20	27,5	01.09.2011	08.09.2011	
32	6,2	02.09.2011	02.09.2011	
21	16	02.09.2011	02.09.2011	
31	18,9	02.09.2011	02.09.2011	03.09.2011
29	13,4	03.09.2011	03.09.2011	
19	16	03.09.2011	03.09.2011	
17	23,3	03.09.2011	07.09.2011	
9	-2		31.08.2011	
1	3,5		01.09.2011	
39	26		05.09.2011	
30	26,6		02.09.2011	

- samples were not processed on the day of arrival
- date of analysis was forgotten/not given
- information given might be incorrect

LabID	E. Coli	Coliform	Standard	Description Method
1	36	40	ISO 9308-1	
2	0	0	ISO 17025	TBX agar (44°C and VRBA(37°C) 24 h
3	22	22	Colilert	24h
4				
5	1800+			McKonkey broth 37,5°C 20 h
6	>300	>300	ISO 9308-1	Tergitol7 37°C 21 h Indole and Oxides
7	32	32	ISO 9308-1	37°C over night on mLGA
8				
9	20,7		Colilert	35°C 18 h Colilert-18 media
10				
11	15000	15000	ISO 9308-1	0.47um membrane filter on Tergitol-7-Agar. Incubated at 37degrees for 24 hours. Confirmed using oxidase and indole.
12	1800+	180	ISO 9308-1:2000	37°C, 24h, Tergitol7 Agar confirmed bi Oxides test and growth´Wh on TBX
13	11	210	ISO 9308-3	1. Lauryl tryptose broth medium incubated at 35ºC for 48hrs; 2. Brilliant green bile broth medium incubated at 35ºC for 48hrs; 3. EC broth medium incubated at 44.5ºC for 48hrs; 4. Tryptophan broth medium, incubated at 44ºC for 24hrs.
14	201	201	Colilert	Incubation temperature @35ºC (In temp 34.8ºC - Out temp 34.9ºC); 21h
15	18		ISO 9308-1	diluent;1/4 strength Ringers solution; Membrane Filtration Method, using Lactose TTC agar,confirmation tests: Oxidase negative & Indole positive
16	18	18	ISO 9308-1:2000	100 ml of sample was filtered on a cellulose acetate membrane filter and placed on Lactose TTC agar for 24 hours at 37oC. Typical colonies were picked up to perform oxidase test and also inoculated in tryptophan broth and incubated at 44oC for 24 hours. Indole test were performed on each tube to confirm presence of E. coli.
17	425	425	ISO 9308-1:2000	24h at 37°C using Tergitol with TTC supplement
18	6	6	ISO 9308-1:2000	Tergitol-7 at 37oC, for24h, Tryptophan broth at 44oC for 24h and Kovacs reagent
19	152	152	ISO 9308-1	Lactose TTC agar, 24 hrs at 37 oC
20	105.000	0 other than E. coli	chromocult coliform agar	incubate at 37°C +-1 for 24h for e. coli in chromocult coliform agar

LabID	37°C	22°C	Method	
1	54	51	ISO 6222	
2	40	43	ISO 17025	Nutrient agar 24 h
3	54		ISOI 8199	37°C
4				
5	2.974			Plate count agar 37,5°C 20h
6	113	151	ISO 6222	Plate count agar
7	75		ISO 6222	Incubated over night ad 37°C on least Extract agar
8				
9	148		IDEXX multiple Enzyme technology	35°C 48 h simplate multiples Media
10				
11	630		APHA-AWWA-WPCF 17th Edition	Pour plate using Standard Plate Count Agar. Incubated at 37 degree celsius for 48 hrs.
12	950	970	ISO 6222	Yeast Extract agar, 37°C 48 h; 22°C 5 days
13	20.000		APHA 9215	Plate count agar and buffered peptone water were used and the sample was incubated at 35ºC for 48hrs
14				
15	45	48	ISO 8199:2005	36°C: Pour Plate Method, using Standard Plate Agar; 22°C:Pour Plate Method, using Nutrient Agar
16	45	51	ISO 6222:1999	37°C:1.0 ml of sample is inoculated in about 30 ml of molten water plate count agar in a petridish, the content is well mixed and allowed to solidify. Incubation temperature 37ºC + or - 2ºC for 48 Hors;Incubation temperature 22oC + or - 2oC for 72 hours
17	124	127	ISO 6222:1999	48h at 36°C yeast Extract agar;
18	57	61	ISO 6222:1999	37°C nutrient agar 48 h; 22°C: nutrient agar 72h
19	580.000	480.000	ISO 6222	36°C: Yeast extract agar 48h; 22°C Yeast extract agar 72 h
20	10.200.000		NF-V-08-051:1992	Incubate at 37°C +-1 for 48 +-3 h in plate count agar

21	90		TES/MIC/1m/16 (ISO 8199:2005)	media standard plate count agar; 37+-1 °C 48h
22	61		9215	Pour plate method used and plates made using yeast extract agar then incubated at 37ºC for 48 hrs.
23	36		APHA 1993	Plate Count Agar (OXOID), incubation temperature 35±2^{0}C for 18-24 hrs.
24	47		APHA	Pour plate method, using Tryptone Glucose Extract Agar; 35+-2°C
25	innumerable		AWWA 9215 B;Heterotrophic Plate count (Pour Plate)	48 Hours, 35.0 ºC, Yeast Extract Agar, Merck
26	53		APHA, AWWA and WPCF, 1989	Heterotrophic Plate count (Pour Plate);48 Hours, 35.5 ºC, Yeast Extract Agar, Merck
27	426	450	ISO 6222:1999	Yeast extract agar; 37°C 48h; 22°C 72h
28	2.800		ISO 4833-2003	Plate Count Agar at 30 degrees for 72 hrs -Aerobic Incubation only
29				
30	30.000		TZS118:2007	PCA at 30˚C - 72hours
31	29.000		Laboratory manual in general microbiology By Harold J. Benson ISBN 0-07-231888-0 (2002)	Plate count agar Trypton Glucose Extract AGAR spread (TGEA); 24h; 35°C
32	205.000		EAS 217-2:2008	PCA at 30˚C - 48 h
33	512	310	NF EN ISO 6222	Incorporation on Tryptone soy agar After 48 hours of incubation: - in 36°C for the mesophilic microorganisms, - in 22°C for the psychrophilic microorganisms
34	54	60		24h Soybean casein digest agar
35	21			Incubation at 36°C/24 hours; m-FC-Saetorius media; Membrane Filters
36	52		spread plate	48h plate count agar
37	136		SAZS 560:1997	Samples diluted and inoculated using pour plate method in Plate Count Agar at 35°C 24-48h
38	41		ISO 4833	Plate Count Agar Colony Count Technique @ 37 OC for 24 – 48 ± 2 hours
39	10.000	1550	ISO 6222	24h Gelose tryptonee au Soya (TSA)
40	660	380	ISO 4833-2003	The samples were incubated at 37°C and 25°C for 48h using plate count agar (PCA)

Conclusions

- calculation of an assigned value possible - major step forward
- packaging and transport needs to be redesigned
- laboratories report standards and methods that do not match