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**4<sup>th</sup> 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 (Algorithm 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 4<sup>th</sup> 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 4<sup>th</sup> microbiological PT round in 2011. Previous workshops on preceding PT rounds have been held at Windhoek (Namibia, 2010), Mahe (Seychelles, 2009) and Kampala (Uganda, 2008). The reports are available from <http://www.sadcmnet.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 assembly of the SADCWaterLab Association elected a new project management committee. The new PMC faces the task of improving 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 faecal 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 approximately 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.
- **Madagascar** (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 chemistry 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 been 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 pesticides.
- **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 trainers (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 ( $\beta$ -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](http://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 analyzed
- check label: source of sample sampling date; parameters needed; type of water;

preservation (chlorinated water)

- let samples reach room temperature
- check measurement 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 independent 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 than 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).

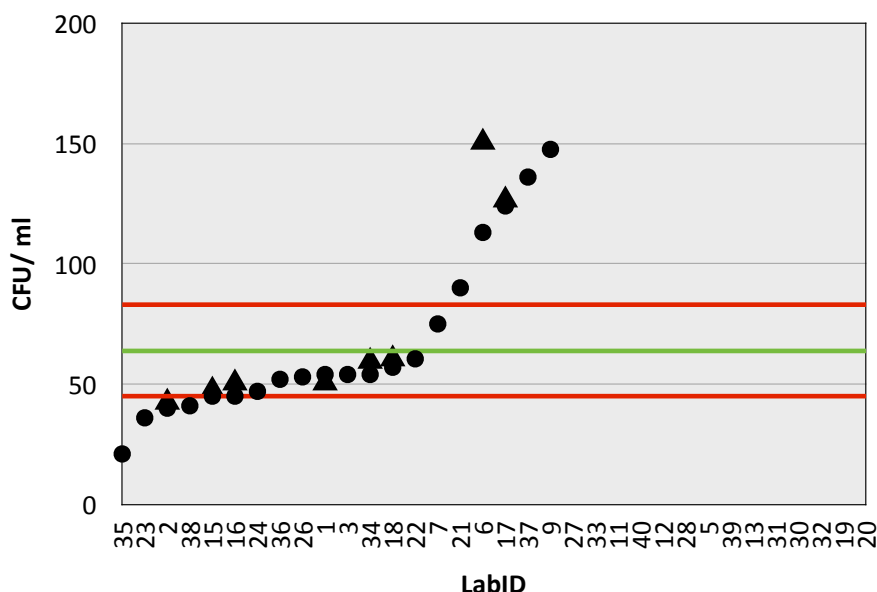
### E. coli / coliform bacteria: evaluation and assessment

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 did 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 emphasis 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 possibility 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 microbiolgy 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	
• <b>What topics would you suggest for further training</b>	
• 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	