



**2nd Microbiology PT
Evaluation Workshop
within the SADC MET
Proficiency Testing Scheme
for Water Testing Laboratories**

Report on the 2nd Microbiology PT Evaluation Workshop within the SADC MET Proficiency Testing Scheme for Water Testing Laboratories

Victoria, Seychelles, 16-19 November 2009

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Summary

17 participants representing organizations from 9 different countries met in Victoria, Seychelles, at the evaluation workshop of the 2nd Microbiology Proficiency Testing round. In August 2009 Uganda National Bureau of Standards for the second time provided microbiological test samples for proficiency testing of water laboratories within the SADC and EAC region. 11 laboratories participated in this PT round.

It seemed that promotion of the PT was not satisfactory as only emails were used as means of communication and that system has failed badly. All participants were encouraged to get in touch with the PT provider if they do not receive a notification in the future. Moreover the PT provider will have to use more means to promote the scheme including the SADC MET website and the local coordinators.

One of the major problems encountered in the first PT round were unfavorable transportation conditions. The samples are only stable for a short time at 3-10°C. Changes were made in packaging and courier system and great improvements achieved. All samples were received in due time and with satisfyingly low temperatures. Unfortunately the number of participants was very low. Therefore it is difficult to assess whether the chosen transport system will work for all countries within the SADC region. But it is a very encouraging development.

Preparation of the PT samples seemed to be satisfying but there is still room for improvement in terms of stability of the samples and consistency of the homogeneity and stability data.

Due to the low number of participants it again was not possible to have a statistical evaluation just as in the first PT.

Again all laboratories were asked to report not only the number of microorganisms they had detected but also additional information on the methods used. Sometimes the standards quoted and the details given did not match. Therefore part of the workshop dealt with the question of how to use and interpret standards.

To give some practical advise in this aspect total plate count methods were discussed in working groups and in a trouble shooting session. The opportunity for networking among the participants and sharing experiences seemed to be quite useful.

All participants were really interested in the topics discussed and valued the workshop as helpful for improvement of their laboratory work.

Introduction

The workshop served to discuss the evaluation of the second microbiology proficiency testing scheme for drinking water in the SADC and EAC region. A previous workshop was held in Kampala in 2008 after the first microbiology PT scheme. The report is available at the SADCMET website (<http://www.sadcmnet.org>).

This year's workshop was held in conjunction with the 6th evaluation workshop within the SADCMET proficiency testing Scheme for chemical parameters for water testing laboratories.

During previous workshops the SADCWaterLab Association had been formed to enhance cooperation and networking among laboratories and a General assembly of SADC WaterLab Association has been held during the workshop.

Participants

The workshop was attended by 17 participants from the following countries:

Kenya	3
Malawi	1
Mauritius	1
Rwanda	1
Seychelles	6
Tanzania	1
Uganda	2
Zimbabwe	2

A complete list is given in annex 1.

Workshop Programme

Monday, November 16th 2009

Welcome, Opening, Report of the PT provider (J. Kwesiga, Uganda National Bureau of Standards, UNBS) Evaluation of PT results

Tuesday, November 17th 2009

Evaluation of PT results continued, assigning reference values (K. Luden and M. Linsky NMISA), working groups on total plate count methods and troubleshooting session on TPC methods, introduction to SADCWaterLab association (D. Masuku, SADC Secretariat)

Wednesday, November 18th 2009

Working with standards, promotion of microbiology PT, discussion on PT brochure, general assembly of SADCWaterLab Association

Thursday, November 19th 2009

Visit at laboratories of Seychelles Bureau of Standards (SBS)

Welcome and Opening

A welcome to all participants of the microbiological and the chemical PT workshop was given at the International Conference Center of Seychelles by Mrs. Amy Quatre, Chief Executive Officer Seychelles Bureau of Standards and Mrs. Marise Berlouis, Principle Secretary for Industries both emphasizing the importance of interlaboratory comparisons. Kathrin Wunderlich representing PTB also welcomed the participants on behalf of the main sponsor PTB. Donald Masuku as representative of SADC Secretariat in the opening ceremony emphasized that participation in a PT scheme is not sufficient to ensure high quality of results but needs to be accompanied by learning from mistakes and conducting corrective actions.

Experience and report of the PT provider

Jacqueline Kwesiga of UNBS reported about her experiences with the 2nd round microbiology PT. She described the preparation of the second round.

In April and May two notifications were sent by email to all participants of the first PT and all participants of the 1st microbiology workshop. Unfortunately only 11 laboratories registered for the second Microbiology PT for drinking water. As transportation times and temperatures were critical aspects in the first PT a different approach was used this time. Samples were distributed using three different pathways. For participants within Uganda UNBS staff transported the samples. For east African countries samples were packed into a cardboard box insulated with polystyrene foam and hard shell ice packs. For South African countries packaging material was purchased from the courier (DHL) and the transport cold chain was used. This rather complicated system seemed to have worked. Sample temperatures at arrival were all below 10°C as required for sample stability and all packages have been delivered within three days.

The full presentation can be found in annex 2.

Evaluation of the 2nd Microbiology PT

The second round microbiology proficiency testing scheme for drinking water analysis was announced by email in April to all participants of the previous workshop and participants of the first PT. A second notification mail was sent in June. Unfortunately the mail system has failed badly. A lot of people did not receive the mails although they were on the mailing list with a correct address. Nevertheless 11 laboratories from 6 different countries registered for participation. UNBS as the provider had switched to DHL as courier and samples were delivered after three days at the latest. Sample temperatures reported at reception of the packages had to be measured in an extra bottle provided with the samples. Reported temperatures lay between 0 and 6°C which was a great improvement. 10 out of 11 laboratories reported their results by mail or fax.

The results of the PT are summarized in table 1 below.

Table 1: Results of participating laboratories for sample A (Total Coliforms/*E. coli*) and sample B (Total Plate Count)

Lab ID	Delivery date	Date of analysis	Temperature at arrival °C	Sample A		Sample B	
				<i>E. coli</i> CFU/100 ml	Coliform bacteria CFU/100 ml	TPC CFU/ml temperature 1	TPC CFU/ml Temperature 2
2	6	6	6	0	0	150	
3	4	4	3	0	28	12	14
4	4	5	3	0	30	13	14
5	4	5	5	0	Pos	45	
6	4	5	5	0	16	32	
7	5	6	3		63	>300	
8	5	6	1	0	0	6	6
9	6	7	0	0	0	10	
10	6	6	4	0	0	4	
11	4	5	4	0	7	0	0

Coliform bacteria / *E. coli* (Sample A)

The quality of participants' results seemed to vary quite much. All laboratories reporting negative or zero for *E. coli* gave correct results as sample A did not contain any *E. coli*. The number of coliform bacteria reported was in the range of 0 to 63. As the number of participating labs was too low to reasonably use these results to assign a target value for the number of coliform bacteria the homogeneity testing of UNBS was used for comparison. Homogeneity testing was done two days after dispatching the PT samples. 20 randomly picked bottles of the original PT samples were analyzed under repeatability conditions. The results can be seen in figure 1 below.

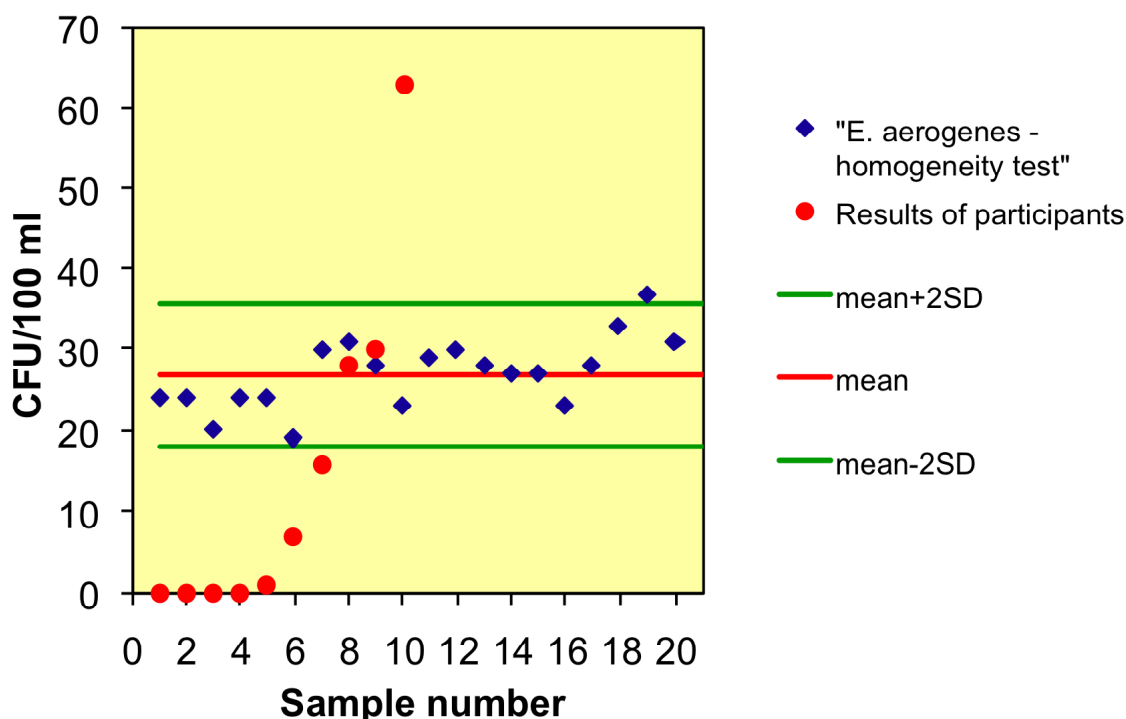


Figure 1: Coliform bacteria sample A; Analysis of homogeneity and participants results. The green lines give the range between mean plus 2 standard deviations and mean minus 2 standard deviations of the 20 test results. These lines are only given for information.

Statistical evaluation was not conducted because of the low number of results and the large range of results compared to the quality control data of the PT provider. All laboratories that did find *E. aerogenes* might be in the correct range. Laboratories that did not report any coliform bacteria should review their procedures. It also has to be taken into consideration that some samples were close to freezing at arrival and this might have led to loss of target organisms during transport.

Total plate counts (sample B)

As for the parameter Coliform bacteria / *E. coli* the quality of the results seemed to vary. The quality control data of the PT provider was also not as satisfactory as for sample A although the same strain and preparation had been used. The PT provider will have to work to further improve the preparation. Multiple test runs during the year will be necessary for that.

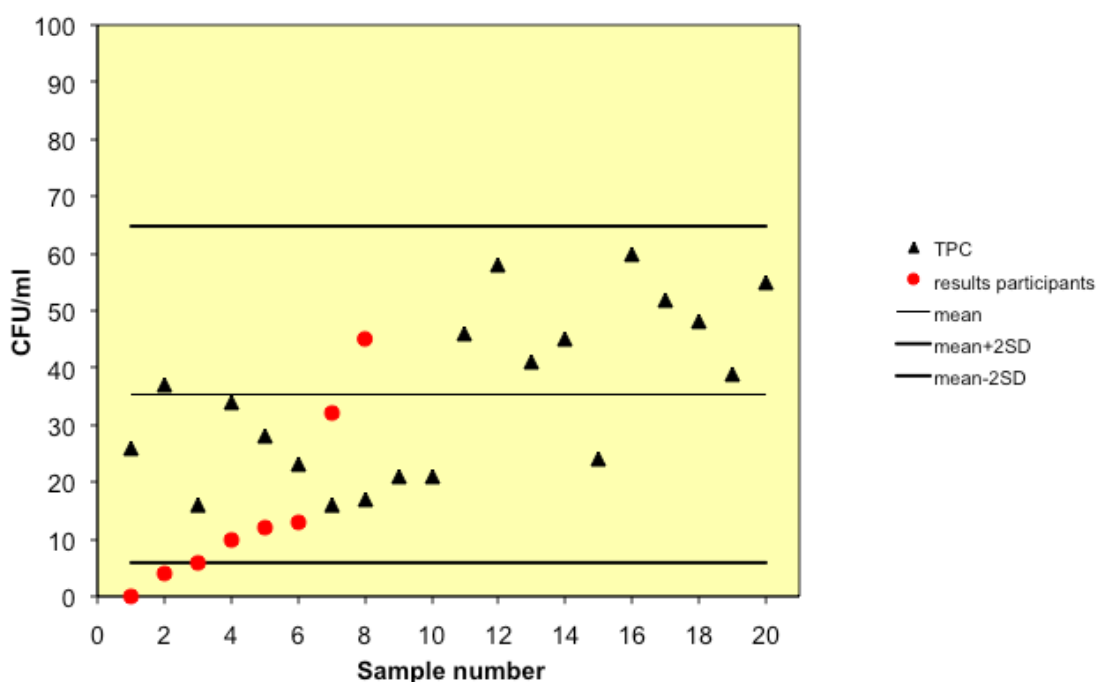


Figure 2: total plate counts sample B; Analysis of homogeneity and participants results. The black lines give the range between mean plus 2 standard deviations and mean minus 2 standard deviations of the 20 test results. These lines are only given for information.

All laboratories but one reported results different from zero and might be in the correct range. As the number of participating labs was too low to reasonably use these results to assign a target value by statistical means the homogeneity testing of UNBS was used for comparison. Homogeneity testing was done two days after dispatching the PT samples. 20 randomly picked bottles of the original PT samples were analyzed under repeatability conditions. The number of Colony forming Units reported was in the range of 0 to >300.

The full presentation on the evaluation of the PT as presented during the workshop can be found in annex 3.

Assigning reference values

The difficulties of assigning reference values in general and special difficulties for microbiological proficiency testing were discussed by Dr. Katrin Luden. As one is dealing with living organisms there are hardly any reference materials that are certified for their numbers. Therefore the most commonly used method is to use some sort of consensus value as target or assigned value. Different ways of assigning target values were discussed during the PT evaluation. Next to using consensus values it might be possible to use data from reference laboratories. There would be the need for a very convincing argument why a certain laboratory is to be believed to perform better than others in order to be accepted widely as a reference laboratory. Therefore this road of action is rarely taken. A better way would be to attract more laboratories to participate in the next microbiology PT round in 2010. Hopefully then a consensus value can be calculated based on robust statistics.

Mare Linsky (NMISA) contributed to this topic by presenting some of NMISA's experiences on assigning reference values for chemical compounds (see annex 4).

General aspects of laboratory performance / Training on standardized methods

All laboratories had been asked to report not only the methods used but also to provide detailed information on media, temperatures and times used. It was quite obvious that sometimes the details given did not match the standards quoted (table 2).

Table 2 Methods and detailed information reported by participating laboratories for sample A (Total Coliforms/E. coli)

Method	Medium	Temp °C	Time
9308-1	Filter membrane		24
1. presumptive	Mc Conkey broth	37	48
2. pour plate	VREA	37	48
9308-1	MLSB		44+-0.5
9308-1	VRB	31	24
RSIS;RS217-3 KS220	VRB		48
Membrane filtration	VR		24
9308-1	VRLB	37	24
9308-1	L TTC	37	24
Membrane filtration	LSB	35	24
Spread plate	Mac Conkey	37	48
7251		37	

Where to find relevant e.g. ISO Standards and how to use them was a topic during the evaluation. A list of ISO methods was provided (see annex 5).

To assist with corrective actions working group discussions were used to come up with a list of aspects to be kept under control and checked when problems arise (see below).

Working group discussions

Discussions on the question how to approach corrective actions for total plate counts were lively. All participants of the workshop got involved in making the list below. One of

the participants took the lead in the discussion and Dr. Luden only intervened when additions had to be made or arguments were not plausible. It seemed that almost everybody had some aspects to contribute but also some things to consider for his or her own work. Below find the final checklist. It is a good help but may not be complete as it is impossible to come up with a list that suits all possible laboratory procedures.

Total plate count methods: steps to keep under control

- Preparation of media (ready constituted media) – pH, expiry date, efficacy, sterility, constituents, weighing, verification of balance (calibration), mixing (conductivity, pH of water)
- Heat to boiling (must not char)
- Autoclaving – pressure, effectiveness (e.g. bacillus capsules, TST strips), time, temperature
- Media tempering to 50°C
- Final temperature below 50°C
- bring sample to room temperature
- Homogenizing of the sample
- Pipetting – calibration
- Sterilization of glassware (cool to room temperature)
- Labeling (no mix up of samples)
- Duplicates might be necessary
- Controlled environment (burner, no open windows, negative control plate)
- Aseptic techniques used at all times
- Dry the medium flask before pouring
- Avoid droplets from outside of sampling bottle
- Pour the agar not directly onto the sample
- Positive control
- Solidification (high evaporation as indication of too high temperatures)
- Invert the plates
- Incubation time and temperatures
- Counting (reading of the plates)
- Using the right magnification (3x? 6x?)
- Reporting / calculation of results – dilution factor
- Reporting in time

Working group discussions:

What benefits did you draw from the 2nd PT evaluation?

The benefits of the evaluation were seen in the opportunity for networking as well as being able to realize mistakes and find ways to improve. The importance of participation in PT schemes was stressed by the participants and the development of a brochure to convince laboratory managers and other decision makers was asked for. The evaluation

served to assess the competence of the laboratory and the test method capabilities. Participants developed confidence for accreditation audits.

- Networking
- Realize mistakes
- Ways to improve/improvement opportunities
- Pass message to managers how important PT is
- Asses the status of competence of lab and/or staff
- Assess test method capabilities
- Confidence building in own capabilities
- Confidence during audits
- Sample more stable/repeatability was OK
- Evaluation of reproducibility was possible

What is the way forward (improvements)?

For the future several requests were stated e.g. the PT provider should arrange for better arrival times of the samples. A lot of samples were delivered quite late in the day so analysis started the next day only. The packages should be labeled clearly for storage conditions. The tracking number should be given to the participants so they know when to expect the samples

- PT provider arrange other times for sample arrival
- Label the package for storage conditions
- Tracking numbers should be given to the participants
- Higher frequency (2x a year) to confirm corrective actions are effective
- Contact names of local coordinators (micro)

How can more participants be attracted?

The participants came up with quite a few ideas that could be considered by the PT provider SADC secretariat and the local coordinators in their attempt to promote the PT scheme.

- Meetings with local coordinators
- Separate local coordinators for microbiology
- More communication/dissemination of information
- Help convince the management (brochure)
- Contact national or regional accreditation bodies
- National accreditation focal points (NAFP) see SADCAS brochure
- TBT enquiry points office
- Initiate formation or contact national lab associations for raising awareness

Introduction to the SADC MET water PT and the SADC Water lab association

As a lot of the participants attended the workshop for the first time Donald Masuku in his function of regional coordinator of SADC MET gave an introduction to The SADC MET water PT schemes and the SADC water lab association. He described the start of the SADC water PT in chemistry 2004 its further development and the forming of SADC water lab association. The association is a regional not for profit organization. Its major aims are:

- To facilitate technical cooperation and collaboration amongst regional labs involved in water testing
- To run a proficiency testing scheme for water analysis
- To provide an organized interface at the regional level between these labs and other SQAM structures involved in conformity assessment issues
- To promote development and harmonization of measurement, test and analytical methods
- Capacity building
- Promote Labs accreditation

Local coordinators have been appointed in each country to coordinate and promote PT schemes at national level for both chemistry and microbiology. Their important role has been stressed.

In 2005 it had been decided that the PT should be extended to microbiology and three people were sent to Germany for training in 2006. At the following workshop in Garborone UNBS was appointed the provider.

The full presentation can be found in the full report of the Microbiology Workshop of Kampala 2008.

The minutes of the General assembly of SADCWaterLab will be made available by the SADC secretariat on the SADC MET website.

Laboratory visit at Seychelles Bureau of Standards (SBS)

All participants had the possibility to visit the microbiological and chemical laboratories of the Seychelles Bureau of Standards.

Evaluation Questionnaire

An evaluation questionnaire was distributed for the microbiology workshop to be answered by the participants (annex). 16 questionnaires were handed back. The questions and answers are given below:

How do you judge:	Very good	good	fair	poor	very poor	Mean
	1	2	3	4	5	
The venue of the workshop	7	9	1	0	0	1.6
The hotel (accommodation)	0	5	4	0	0	2.4

How do you judge the different parts of the workshop	Very useful				Not useful	Mean
Evaluation of the PT	12	5	0	0	0	1.3
The working group discussion on TPC	12	5	0	0	0	1.3
The troubleshooting session (TPC checklist)	4	11	1	0	0	1.8
The presentation on assigned values (NMISA)	3	9	14	0	0	2.1
SADCWaterLab assembly	5	10	1	0	0	1.8

Did the workshop fulfill your expectations? Yes/No/Partially If no or partially please explain. Answers Yes: 16 No: 0 Partially: 1

Explanation: I hoped to learn in depth (training) on at least some aspects of the PT (for example statistical analysis) with some practicals.

What were the most important topics to you? Number of participants listing the topic

- Working group discussions on TPC and troubleshooting session 9
- Evaluation of the PT 3
- Discussion on the problems with the methods (fit for purpose method) 3
- Presentation on assigned values 2
- Working with standards 1
- SADCWaterLab Association 1
- train the trainer workshop 1
- Aspects of results variance 1
- Method variance 1
- All topics discussed were valuable 1
- Discussion of results from different labs and the ISO standards by Dr. Katrin Luden. 1

What benefits did you draw from the workshop?

- The workshop has helped us to improve our methods in the lab. Assess our performance.
- I know position of my labs capability.
- Know techniques in TPC testing
- Networking
- Challenges and benefits of the PT scheme
- Different methods and standards that can be implemented and others are not applicable
- A lot of it

- Networking
- Better ideas on opportunities for improvement
- Quite ... And learnt a lot
- It increased my knowledge on different standards and methods used in the PT
- The workshop helped me to increase my confidence and to see my weak point.
- Awareness of the PT scheme and the importance of the PT in the laboratory.
- That the method my lab is using corresponds to other participants' method.
- How to implement corrective action when I get back and implementation on ISO/IEC 17025 in microbiology.
- There is need for effective marketing of the PT.
- Wise after the event. We know our weakness and way to improve our test methods.
- I networked with other members from other labs in Africa and from them I will be able to obtain useful information that will be beneficial to my lab.
- Improve my working methods. I learn from my mistakes the importance to pass the message to management about PT and take participation.
- Confidence building and chance to assess lab competence
- Opportunity for improvement in some areas
- Network
- Confidence in the methods used at our local labs in Uganda
- Correction of mistakes
- Confidence to answer questions from audit in accreditation.
- A lot of benefits but most important that in the testing world I am not alone.

Concluding remarks

- The microbiology PT needs to attract more participants through better promotion of the scheme.
- Although sample preparation was considered satisfactory there is still the need for improvement.
- Most participating laboratories had some inconsistencies in their reported results. Either they did not find microorganisms in the required range or gave inconsistent information on the methods used. Therefore corrective actions should be conducted.
- A brochure for promoting the microbiology PT scheme was discussed and will be printed and distributed by SADC secretariat.

Report prepared by Dr. rer. nat. Katrin Luden

Aurich, 04.04.2010