Report on the 1st Microbiology PT Evaluation Workshop within the SADCMET Proficiency Testing Scheme for Water Testing Laboratories

Kampala, Uganda, 11-12 December 2008

Prepared by Dr. rer. nat. Katrin Luden

Summary

The workshop dealt with the evaluation of the first SADCMET microbilogy PT for water testing laboratories. Samples for the parameters E. Coli/Coliform bacteria and Total Plate Counts were prepared and shipped by UNBS in October 2008. Due to the wide range (several log-scales) of the results this first PT could not be evaluated by statistical means. This could either be due to insufficient stability of the sample taking into account the long transportation times or due to bad performance of the participating laboratories.

A closer look at the results and information on methods used in the analysis of the PT samples revealed that there might be a need for improvement in quite a number of laboratories. Therefore the individual results have been discussed extensively and an additional training on Total Plate Count methods and E. Coli/ Coliform methods has been conducted. These parameters have been identified by the participants as the most important parameters in the workshop of 2006 in Garborone and confirmed in the recent workshop by the microbiologists.

The participants were very interested in the experience and use of the PT and expressed the need to go on with the PT.

A lot of information has been gathered from the workshops working group discussions to help UNBS as a PT provider to improve the quality of their performance as a provider. Problems with sample transport and packaging have been discussed. Stability of the samples under optimized conditions at UNBS seemed to be satisfactory. Nevertheless some improvements in the preparation process and its documentation must be attempted and have been discussed at the laboratory.

The outcome of this first microbiology PT stresses the need for further microbiology PT schemes on the side of the participants. The first important step toward an independend microbiological PT scheme within the region has been taken but it will be crucial for this type of liquid samples to get the transport conditions and times under control to make it work.

Introduction

The workshop held in Kampala was the first one in the SADCMET region covering a microbiology proficiency testing scheme provided in the region. In previous workshops dealing with the chemical PT scheme the need for a microbiological one was stressed.

Therefore in 2006 three people were sent to the Niedersächsisches Landesgesundheitsamt in Aurich which is the german PT provider for microbiological parameters in drinking water. At the workshop in Garborone in December of the same year it was decided that an attempt should be made to establish a regional PT system with UNBS as provider using the german system of liquid samples. The PT schemes should be offered to laboratories within the SADC and the EAC region and the trained colleges from Tanzania and Zimbabwe should act as backup in case UNBS can not longer provide the PT.

The workshop was preceeded by the 5th evaluation workshop on Proficiency testing for water laboratories (Chemistry) and a day of training on management demands of the ISO 17025 and VIM. Some of the participants stayed on to the microbiology workshop.

Participants

The workshop was attended by 29 participants from the following countries:

Botswana	2
Ethiopia	1
Kenya	3
Malawi	1
Mauritius	1
Namibia	3
Rwanda	1
South Africa	3
Swaziland	1
Tanzania	2
Uganda	8
Zambia	1
Zimbabwe	2

A complete list is given in annex 1.

Programme

Thursday, December 11th 2008

- **Welcome and Opening** (Patricia Ejalu)
- **Experience and report of the PT provider** (Patricia Ejalu)
- **Introduction to the SADCMET water PT and the SADC Water lab association** (Donald Masuku)
- **Evaluation of the 1st Microbiology PT** (Dr. Katrin Luden)
- **Working group discussions on the implementation and performance of the first microbiology PT:** (all participants)

Welcome and Opening

A welcome was given by Patricia Ejalu of Uganda Bureau of Standards (UNBS) on behalf of the PT provider and host of the workshop. All participants introduced themselfes.

Experience and report of the PT provider

Patricia Ejalu reported about her experiences with th 1st round microbiology PT. She described the precedings of the first round. First of all it was not easy to get all the equipment, media and packaging nessessary for the preparation of the samples. She expressed her gratitude to PTB for sponsoring the refrigerated centrifuge needed. Three trial runs of preparing samples had been conducted between November 2007 and July 2008.

The first notification of the PT was in March and 25 laboratories registered for participation. Samples were shipped in September and some problems were encountered with the packaging. A cardboard box lined with styrofoam had been used. Two sterile bottles filled with chilled samples one hardshell ice-pack and one softshell ice-pack had been used to keep the temperature during transport as low as possible. In one of the packages the softshelled one had burst.

Most of the communication with the participants had gone through email and that seemed to have worked quite well.

She listed the most challenging points for UNBS as PT provider:

- Communication with the local coordinators
- Stability of the sample
- Temperature regulation (very high counts)
- Feedback from the participants (limited information on form)
- Courier service

The full presentation can be found in annex 2.

Introduction to the SADCMET 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 SADCMET gave an introduction to The SADCMET 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.

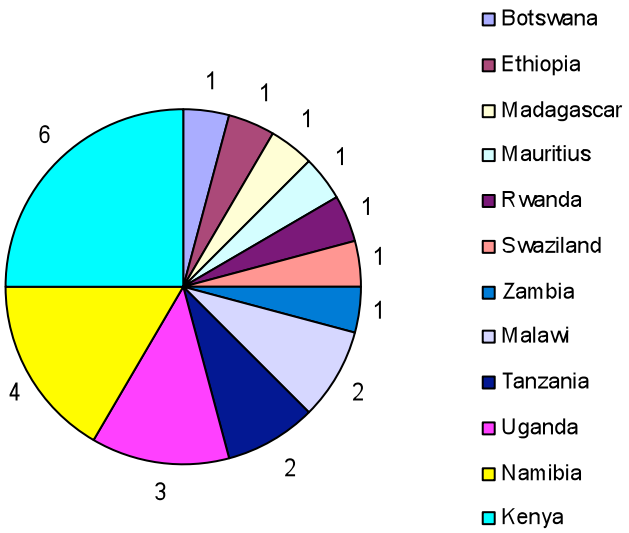
In 2005 it had been decided that the PT should be extended to microbiology and three people were sent to Germany for training in 2006. At the following workshop in Garborone UNBS was appointed the provider.

The full presentation is given in annex 3.

Evaluation of the 1st Microbiology PT

Mrs Luden explained in detail the evaluation of the PT: An introduction to the german microbiology PT for drinking water was given and the difference of handling liquid rather than freeze dried samples as PT material stressed. Stability of the liquid samples is possible in case sample temperature can be kept below 10°C. This might be difficult in the southern african region taking into account transport across long distances and borders as well as high outside temperatures. UNBS will have to optimize packaging and courier system in order to meet those conditions. There are two major benefits of the liquid samples: a) preparation is possible without highly sophisticated special equipment and b) it is much closer to a real sample than any kind of freeze dried material.

24 Laboratories registered for the first microbiology PT scheme provided by UNBS

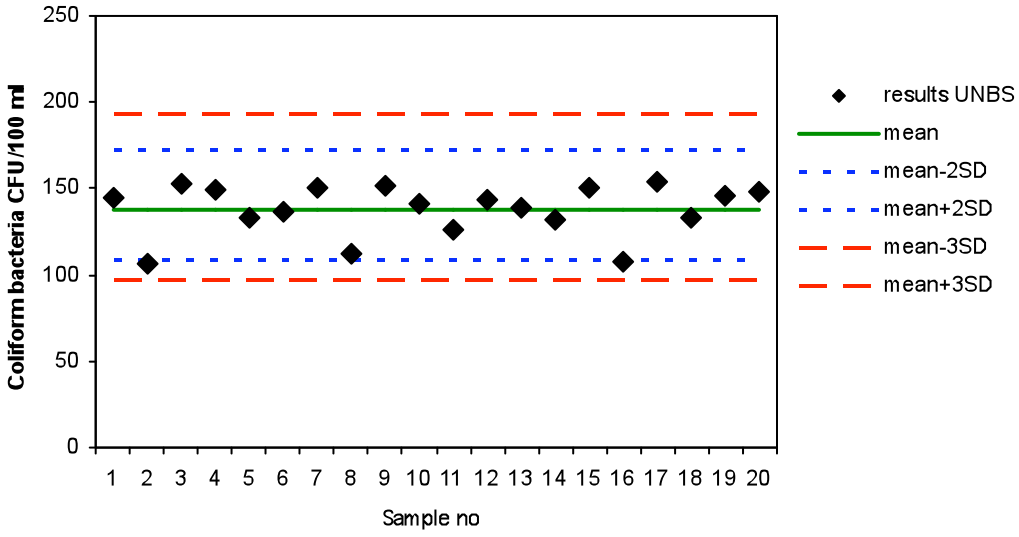


Two samples were prepared for each laboratory. Sample A for the parameter *E. coli*/coliform bacteria and sample B for the analysis of Total Plate Counts (TPC). This was consistent with what was decided in Gaborone 2006. Also the samples were prepared in a manner that simulated drinking water. Feedback from the participants showed that the accompanying letter did not give sufficient information of what type of

water should be expected. This is important information for a lab to decide whether dilutions might be needed.

For distribution samples were handed to the courier Sky Net regularly used by UNBS. Some of the samples were only recieved after several days meaning that temperatures of the samples were high and not under control. This might have given the strain time for growth during transport. In order to optimize the distribution it is crucial to know the exact arrival date of the samples. So the provider will ask all participants to confirm receipt of the sample imediately.

Homogeneity testing of 20 bottles randomly picked and analyzed at UNBS showed that in this aspect the preparation of the sample was satisfactory. Stability testing carried out until day 9 after shipment showed that under refrigerated conditions the sample preparation was stable. Stability for up to 10 days is what is usual for samples prepared by NLGA in Germany as well. Therefore the preparation of the samples seemed to have been quite good which is an important step towards establishing a successfull and reliable microbiology PT system.



There was a wide range of results reported for sample A**:** Coliform bacteria

LabID	CFU/100ml
1	22.000.000
2	106
3	0
4	22.500
7	276
9	>300
10	3.000
11	1.600
12	1.425.000
13	3.000

LabID	CFU/100ml
14	17
16	1.150
17	>300
18	46.000
20	210
21	18.000
22	>150
23	450

No statistical analysis was applied because of the wide range of the results. There are two most probable causes:

a) growth of the used strain prior to analysis or

b) bad Lab-performance

From the data collected it is impossible to say where the main problem was. Nevertheless a closer look at the methods stated in the report sheet and the reported result showed that they were not always consistent. It seems that at least some of the laboratories should check their methods and laboratory proceedings.

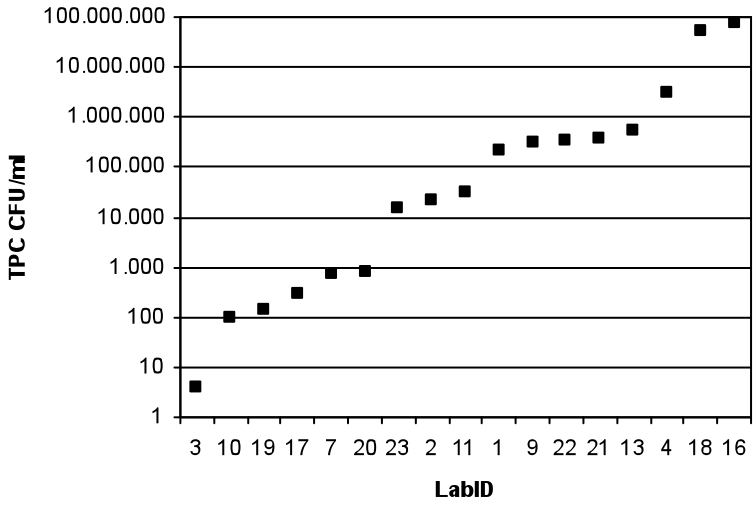
All but one laboratory did find coliform bacteria indicating that the strain is pretty stable at higher temperatures.

Sample B contained the same strain and was spiked from the identical stock solution into the same medium as sample A. And although homogeneity and stability tests had very low counts due to the use of dilutions used it is reasonable to assume that these characteristics were similar to sample A. The concentration of *E. coli* in the sample was most probably approximately 100 CFU/ml. But the results of the participants gave the same picture as in the other sample. And again no statistical evaluation was used.

Results for sample B: Total plate count

LabID	CFU/ml	LabID	CFU/ml
1	210.000	16	78.000.000
2	21.700	17	>300
3	4	18	52.000.000
4	2.950.000	19	135
7	706	20	842
9	323.000	21	370.000
10	100	22	350.000
11	31.000	23	15.000
13	550.000		

Assuming that the concentration was originally in the range of about 100 CFU/ml the mean of the participants results would have been 1000 times higher.



There were several problems encounterd in this first microbiology PT:

- Packaging only held the temperature for 24 hours.
- Homogeneity and stability tests had very low counts due to the use of dilutions used.
- Several thing should have been asked in the reporting sheet:
    - Date of reciept
    - Temperature at the time of receipt
    - Seperate results for *E. coli* and Coliform bacteria
    - Seperate results for TPC at two different temperatures

The overall impression was that UNBS had a good start but there is also still a lot of things to improve. To identify problems an help improving the PT scheme the following questions have been discussed in three working groups. Questions and answeres are listed below. The full presentation of Dr. Katrin Luden is given in Annex 4.

Working group discussions

All participants were asked to answer the following questions after discussing them in three groups:

How did you learn about the microbiology PT?

- from the local coordinator
- at the Botwana workshop 2006
- at the Tanzania workshop 2007
- from the regional coordinator
- from the PT provider

Were you satisfied with the announcement and the information you got from the provider?

- Yes: exiting that a cheap alternative to other PT systems exists
- Yes: information in the announcement was satisfactory
- No: own initiative had to be taken
- More clarification on nature of the sample (bottled, raw water) should be given as well as information on necessary dilutions storage conditions; a larger sample volume would be better

Did you have any problems with the registration

- No

What was the role of the local coordinator?

- Contact the local labs to aquire participants

- Distribute information
- Assist with registration
- Follow up payment

Were there any shipment problems?

- Box damaged, bursted icepack
- Delay in delivery up to 10 days
- Sample temperature 25°C
- Only one icepack
- Suggestions: - Label keep at 4°C, more detailed information about shipment attach a hazard sign
  - put information on the content of the box (non hazardous testing material, not intended for human consumption)
  - look for most reliable courier company in all countries

Did you encounter any reporting problems?

- Email and fax was used and worked well
- Guidance on how the reports are to be filled has to be more elaborate (no > or <)
- TPC: two results should be reported if using the ISO method
- TPC: sample might be used for repeated analysis by several analysts allow for reporting those results
- Space for comments was too small
- Only coliforms was requested on the results sheet but coliforms and E. Should have been analyzed
- For MPN method it was ambigous
- Report is still missing

Did you have any problems with payment/costs?

- Swaziland does not have standard charted bank
- Internal lenghty procedure
- PT provider needs payment invoice

Were you satisfied with the organization of the Proficiency testing scheme?

- Delay of shipment from July to September was unsatisfactory
- Packaging inadequate, use of cool box
- More volume is wanted for multiple analysis
- PT provider should alert coordinator and participants imediately after shipping so the samples can be expected and analysed right away

Friday, December 12th 2008

- **Training on standardized methods** (Dr. Katrin Luden)
- **Working group discussions:** (all participants)
- **Lab visit UNBS** (all participants)

Training on standardized methods

The results reported in the first PT round varied greatly. Fortunately the participants had been asked to give information on the methods used in analysis of the PT samples. Therefore a training session was conducted by Dr. Katrin Luden concentrating on general aspects of using standardized microbiological methods.

The main focuss was on consistency of the given information and the results obtained.

Table of results and reported method used for sample A coliform bacteria:

CFU/100 ml	method
22.000.000	**pour plate, brilliance chromogenic selective Agar (Oxoid), incubation at 36°C**
106	AWWA 9222D Membrane filtration; feacal Coliform procedure; **44.5 °C**; Biolab m-FC agar HG000C92.500; E. coli absent by SABS 221-1990) Inocculation in Tryptone water with possible E. coli and incubate at **44.5°C** for 24 hours Confirm with Kovacs reagent for presence/absence
0	MFM ISO 9308-2, 44°C; MLSB
22.500	MF mEndo Agar LES (Difco); 35 +-2 °C
276	BMM-S11-08; Mc Conkey agar (Oxoid); 30°C
>300	ISO 9308-1; 37°C; Lactose TTC agar
3.000	**MF ISO 9308;37°C M-Endo agar les**
1.600	MPN; 35°C Lauryl Tryptose broth; E. coli present isolated on Eosin Methylene Blue (EMB) agar; EC broth at 44.5°C; Methods described in Standard Methods for the examination of Water and wastewater 20th edition 1998
1.425.000	**Colilert-18**
3.000	
17	
1.150	standard methods 20th Edition MF; 9125D; 44.5°C; MFC Agar, Brilliant green bile; lactose broth
>300	violet red bile agar (unleserlicher Rest)
46.000	ISO 9308-1; 37°C; MPN; Lauryl-sulphate broth, brilliant green bile broth
210	**ISO 9308-1:2000; 37+-1°C; Violet red bile Agar**
18.000	pour plate violet red bile agar; 37°C
9.500	MPN; 36°C 24h; Lauryl sulphate broth/brilliant greenbile broth

Some of the points discussed were:

- It is not possible to actually count a number higher than several hundred/ml in a pour plate method. That would amount to 50.000 CFU/100 ml at the most. ISO 8199 states that if you have more than 300 colonies on a plate your result is so biased by intercolony interferance that you are supposed to give >300 as a result. The biggest effect on bias might have the diminished nutrient availability and therefore one might underestimate the true number of culturable organisms.
- Using Indole production or growth at 44.5°C as a characteristic of *E. coli* to distinguish between other coliform bacteria and *E. coli* you should use a broth and a water bath for incubation. The tolerance of the termperature control should be set at 0.5°C at the most because even E. coli will stop growing or die at temperatures higher than 45°C and other organisms might grow if the temperature is not high enough.
- Using an ISO-method like ISO 9308 means using the exact media listed there M-Endo les is not listed in that ISO standard. The main purpose on using standardized methods is that results become comparable. These methods are lokked at as validated. If you want to use them you only have to do quite little to show that they are working at your lab. You do not need a full scale validation study. If somthing is changed you have to validate that new method and you should state that you are using a method modelled after ISO...
- Colilert-18 is a most probable number method that does not give results as high as 1.425.000 CFU/100ml without any dilutions. Dilutions are not mentioned
- There is no good explanation for all the high results the transport medium does not contain any nutrients. Even if the strain can grow at higher temperatures it should only double once or twice but not thousends of times. On the other hand it is not plausible that so many labs have major contamination problems. The way of how dilutions are used and numbers are calculated should be checked at all labs.

The parameter coliform bacteria was used to demonstrate that a change in the method can lead to very different results. The german legislation concerning this parameter was used as an example.

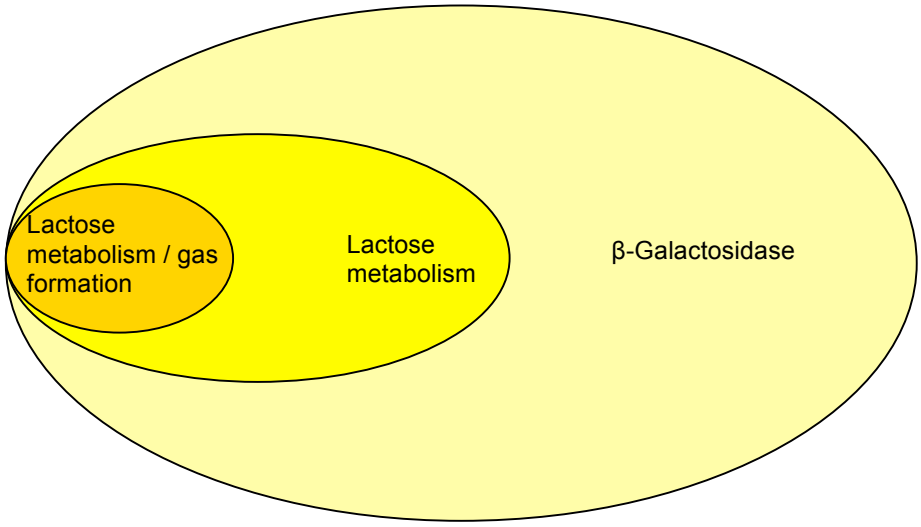
Coliform bacteria are not a taxonomic group. This parameter has always been defined by the method used. Originally lactose fermantation to gas and acid was used to describe this group. Later on only the acid production had been looked at and now there is a method that uses only the activity of a single enzymeto characterize the members of this group. Narrowing down the characteristic from the whole biochemical pathway of lactose fermentation to just one part of it to just one enzyme activity widens the range of organisms falling into that group.

There are several problems if there are different methods allowed for analysis of coliform bacteria:

- Test principles are not identical
  - equivalence considers mean of results (statistics)
- Coliform bacteria detected by one method might not be coliform bacteria by the other method

- how to deal with the differing results of the two “equivalent” methods e.g. samples are analyzed by different labs
- Independent of the method detection of coliform bacteria results in non-compliance to the EU drinking water directive (limit: 0 CFU/100 ml)

The group of coliform bacteria:



“Historic” ISO 9803-1 Colilert-18

It came to the conclusion that as a microbiologist you have to be aware of what you are doing. Keep in mind the test principle to be able to consider the impact of any change you make. Most microbiological methods are convention methods. Changing a method needs carefull consideration of the consequences. One has to look closely at what methods are suitable for your purpose (is there a mandatory/reference method).

For comparability e.g. export purposes it might be good to use methods from international Standards.

Interaction between filter and nutrient agar might be unfavorable for growth of the target organism

Performance of the material combinations used has to be checked (even if supplied with a certificate) and negative controls are as important as positive controls .

The way forward for the PT provider now is to first finish the report of the 1st PT round. To test packaging material and courier systems in order to improve the cooling capacity.

The 2nd PT should be announced. Meanwhile a look at the stability of the used strain at higher temperatures might give a clue to explain what happend in the first PT.. In order to help the local coordinators in further promoting the PT a leaflet should be prepared.

The full presentation is given in Annex 5.

Working group discussions:

Previously the matter of what kind of PT parameters are needed most was discussed in the chemistry PT workshop. Therefore this was repeated to confirm those needs or change the parameters and matrix for the next PT. All questions were first discussed in three working groups and afterwards the answers presented to all participants.

What are the most important parameters? (priority list)

Total Plate Count

E. coli

E. faecalis

P. aeruginosa, Staph aureus

Vibrio cholera

Salmonella

Sulfitreducing anaerobes

Legionella

Shigella

What kind of matrix/water is most important to you?

Drinking water

Bottled water

Borehole water

Ice (for fish export)

Reclaimed water

Surface water (shallow wells, rivers, etc.)

Swimming pool/bathing water

What is the aim of your analysis? *E.g. monitor drinking water for feacal contamination...*

Compliance to regulations

Monitor contaminatnts (e.g. Cholera)

Promoting trade

Product certification (bottled water)

What are the mandatory standards/legal requirements ... you have to consider? *Give standards, parameters and parametric values.*

TPC containerized 20CFU/ml (method and value KEBS)

Communal water 100 CFU/ml (method and value KEBS)

Surface water 100.000 CFU/ml (Zimbabwe)

Absence of pathogens

Bottled water <10 (ZBS)

EU directive for food industry

WHO guidelind for drinking water

Coliforms drinking water <10/100 ml (TZS/BOBS)

Lab visit at UNBS

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

Summary on conclusions from the first microbiological PT and the workshop

- An important first step has been taken on the way to establishing a regional microbiology PT.
- Sample preparation seemed to be satisfactory concerning homogeneity and stability at <10°C.
- There is need for improvement on several aspects on the side of the PT provider: packaging and shipment have to be optimized, more information has to be given to the participants on what kind of sample they recieve, results sheet should be amended.
- There is need for improvement on lab performance of the participants. The table of results and methods used to abtain them indicated that regardless of the lack of statistical evaluation there most probably is the need for corrective action in several labs.
- A leaflet to help the local coordinators promoting the PT scheme should be designed (Dr. Luden, D. Masuku)
- SADCWATERLAB was introduced to all participants and participation ecouraged to try and establish a microbiological network
- An email-list was prepared to improve communication between the participants and facilitate helping each other

Closure of the meeting

Patrica Ejalu, Donald Masuku and Katrin Luden closed the workshop and thanked all participants for their cooperation.

Evaluation Questionaire

An evaluation questionaire was distributed for the microbiology workshop to be answered by the participants. 16 questionaires were handed back. The questions and answers are given below:

How do you judge:	Very good 1	good 2	fair 3	poor 4	very poor 5	Mean
The venue of the workshop (accomodation and conference room)	6	5	3	1	0	1.9
The content of the presentations	9	6	0	0	0	1.4
The working group discussions	7	7	1	0	0	1.6

How do you judge the different parts of this workshop?	very useful 1	2	3	4	not useful 5	Mean
Evalutation of the microbiology PT	11	4	1	0	0	1.4
Training on standardized methods	7	5	1	0	0	1.5

Did the workshop fullfil your expectations? Yes 15 No 0 no answer 1

The most important topics for me have been: number of participants mentioning this topic

- training on standardized methods 11
- micro PT evaluation 9
- group discussions 4
- ISO 17025 3
- coliform bacteria 3
- report from the PT provider 2
- history of SADCMET Micro PT 2
- reporting system of PT samples 1
- Problems encountered 1
- preparattion of PT samples by UNBS 1
- preparation of PT samples Germany andUganda 1
- methods used in testing and how relevant they are comparing to the results produced 1
- ISO 9308 methods checks 1
- ISO 6222 method 1
- importance to have suitable test methods 1
- framework for regional cooperation for technical labs 1
- discussion on the PT results 1
- discussion of evaluation results and reports 1
- brainstorming session for PT provider (sending problems) 1

- brainstorming on the results obtained by various labs 1
- all topics were useful and important to me 1

What benefits did you draw from the workshop?

- I have been made to the of re-adjusting my methods and use validated methods (standardized) to give reliable results.
- aquired new techniques for testing with micro PT
- a very good understanding of the variation between the different test methods used in different countries and the results produced
- Contact with various microbiology laboratory representatives of the regions this will help us to
- Comparison of different microbiology methods their strength and weaknesses
- All information was valuable and relevant. Personally I have learnt a lot. Workshop must be held in November not December. Annual leaves have to be planned well in advance of December Cause problems if workshop is held in December. Everybody in the institutions wants leave in December In General it will be better to have the workshop in November due to availability of the staff
- I noted down all the information given that the lab could use because I am from a chemical background. Workshop should be held in November.
- preparation of PT samples more knowledge on PT analysis
- added competence to the test methods
- Learned a lot about the use of different test methods more accurately; general problems experienced with PT provider of sample distributions
- Covered a lot of things to be collected (corrected?)
- Understood the theory behind development of a PT sample
- Much is needed to be done by participating labs in improving the test methods and henceforth PT performance
- Mmethod validation should be carried prior to test otherwise the results obtained from different methods may vary. I have learned to compare methods and evaluate their fitness.
- useful information on bacterial analysis
- to be very careful when changing methods