2nd Microbiology PT Evaluation Workshop within the SADCMET Proficiency Testing Scheme for Water Testing Laboratories

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

Victoria, Seychelles, 16-19 November 2009

Prepared by Dr. rer. nat. Katrin Luden

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 SADCMET 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℃. 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.sadcmet.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℃ 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℃ 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 ℃	Sample A		Sample B	
				E. coli 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.

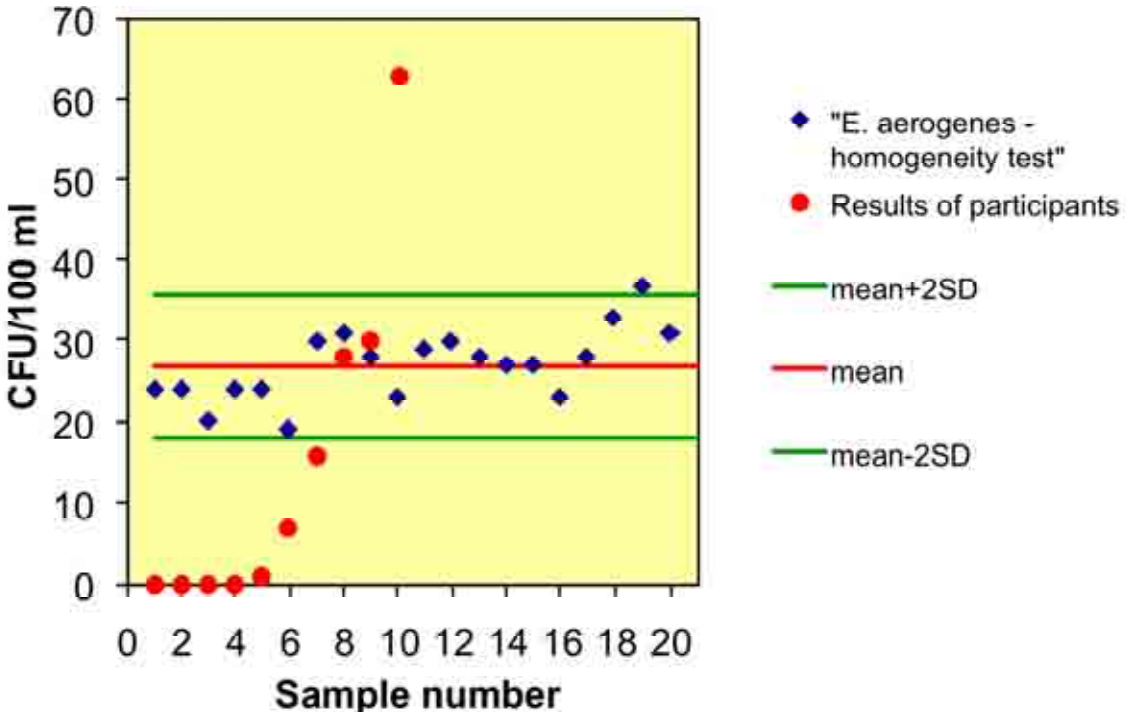

Figure 1: Coliform bacteria sample A; Analysis of homogeneity and participants results. The green lines give the range between mean plus 2 standard deviations and mean minus 2 standard deviations of the 20 test results. These lines are only given for information.

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 lead 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.

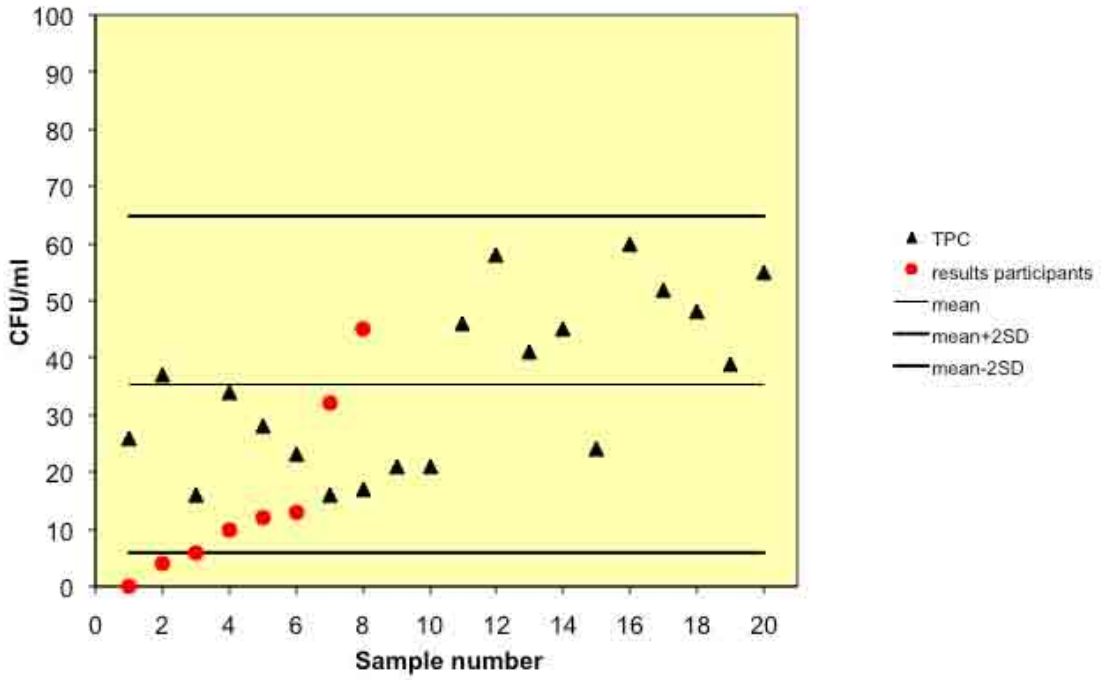

Figure 2: total plate counts sample B; Analysis of homogeneity and participants results. The black lines give the range between mean plus 2 standard deviations and mean minus 2 standard deviations of the 20 test results. These lines are only given for information.

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 ℃	Time
9308-1	Filter membrane		24
1. presumptive 2. pour plate	Mc Conkey broth VREA	37 37	48 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	LTTC	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, constituants, 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℃
- Final temperature below 50℃
- 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 frequence (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 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 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 SADCMET 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 1	good 2	fair 3	poor 4	very poor 5	Mean
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

Participants list evaluation workshop Microbiology PT (drinking water) Seychelles November 2009

MrMs	Surname	First name	Country	Affiliation	email1	email2
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SADCMET WATER PT EVALUATION WORKSHOP

MICROBIOLOGY WATER PT ROUND 2
SEYCHELLES
16TH – 18TH NOVEMBER 2009

Jacqueline Kwesiga
Principal Analyst
Microbiology Testing Laboratory
Uganda National Bureau of Standards.

Background

- Trial Run 1 (21/05/09): Preparation of solution E. ***Escherichia coli*** **NC no 09001 from NCTC (Serotype 01)**
- Trial Run 2 (15/06/09): Preparation of solution E. Used 2 organisms.

1. *Enterobacter aerogenes* NC no.1006 from NCTC
2. ***Escherichia coli*** **NC no 09001 from NCTC (Serotype 01)**

Preparation of 2nd Round

- **1st notification- April 22nd**
- **2nd notification – June 4th**
- Registration – A total of 11 labs
- Participation – A total of 11 labs
- Results received from 10 out of 11 labs

Preparation of 2nd Round - July

- Preparation of solution E – *Enterobacter aerogenes* – 29/07/09.
- Spiking of samples – 1/08/09.
- Bottling of samples - 2/08/09.
- Packaging of bottled samples – 3/08/09.
- Transportation of packages to courier premises – 3/08/09.
- Distribution of samples destined outside Uganda by courier – 3/08/09.

Preparation of 2nd round - Packaging

- Destination East Africa

Included polystyrene foam all round the inside of a cardboard box and 4 hard shell ice packs. Samples were contained in 2 sterile plastic bottles (A and B) and another sterile bottle contained water subjected to the same temperature conditions as A and B. used for determination of temperature on receipt of samples

Preparation of 2nd round - Packaging

Preparation of 2nd round - Packaging

Preparation 2nd Round - Packaging

- Destination Southern Africa

  Packaging was purchased from the courier on there advice. This was owing to the longer distances of travel compared to the EAC countries.
- ` Packaging was Styrofoam and included 6 – 8 hard shell ice packs depending on the size of the Styrofoam packages. Again samples were contained in 2 sterile plastic bottles (A and B) and another sterile bottle (Sample C) contained water subjected to the same temperature conditions as A and B. used for determination of temperature on receipt of samples.

Preparation 2nd Round - Packaging

Preparation 2nd Round - Packaging

Preparation of 2nd Round – Documentation in packaging.

Documentation included:

- A letter of Instructions.
- A results form that included Lab I.D. numbers for each particular lab.

Preparation of 2nd Round - Courier

- A new courier (DHL) was used owing to the limitations of the 1st PT round courier as outlined in the SADC MET water PT evaluation workshop in December 2008 – Kampala.
- The courier was used for transportation of packages to labs destined outside Uganda's borders only.
- For local labs, staff in the UNBS lab delivered the packages – to cut down on costs of courier They were all kept under similar conditions to the ones destined abroad.

Preparation of 2nd Round - Courier

- Courier advised that chilled temperatures (-2^0C – 5^0C) were used on their cargo planes during transportation and in their cold rooms at the various ports.

Feed back

- Tremendous improvement from PT round 1 in the following areas:

1. Speed of distribution of samples.
   - EAC packages delivered within 24 hours.
   - Malawi packages delivered within 48 hours
   - Zimbabwe packages delivered within 72 hours.
2. Temperature regulation of the packages up to the final destination.
   - The temperatures of the samples at the time of receipt as measured by sample C were all $\leq 6^0C$.

Challenges

- Weaknesses identified include:

1. Poor marketing of the local coordinators: The number of participants declined drastically.
2. Lack of payment of PT fees to the PT provider.
3. In some instances the required feedback on the results form was not conscientiously filled in

THANK YOU AND ANY QUESTIONS

SADCMET Water PT evaluation workshop
Microbiology Proficiency testing
2nd round

About me

- Dr. Katrin Luden
- Health protection agency of Lower Saxony/ Germany (NLGA)
- Microbiologist
- PT provider in Germany

Dr. Katrin Luden

Microbiology PT by UNBS

- 2006 training at NLGA in Germany
- 2008 1st PT
  - Problems with transport system (times and **temperatures**) / PT provider
  - Standards not applied properly / laboratories
- 2009 2nd PT
  - ....

Dr. Katrin Luden

Principle of PT scheme

- Liquid samples with living organisms
- **Most important: very realistic samples**

➢ Limited stability (7 to 10 days)

➢ Samples must be at low temperatures

- *Robust statistics*

Dr. Katrin Luden

Participation

- 11 Laboratories (versus 24 in 2008)
- Laboratories from 6 countries participated in this 2nd scheme:
  - Kenya – 3 Laboratories
  - Rwanda – 1 Laboratory
  - Malawi – 2 Laboratories
  - Swaziland – 1 Laboratory
  - Zimbabwe – 2 Laboratories
  - Uganda – 2 Laboratories

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Schedule 2nd PT

- 1st announcement: April 21st by email
- 2nd announcement: June 4th by email
- Dispatch of samples: August 3rd
- Deadline for reporting results: August 14th
- Report of PT provider: end of October
- Evaluation and training workshop: December

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General information

- Courier: DHL
- 100 ml bottles used for all parameters
- Samples resembling drinking water
  - A: E. coli/Coliform bacteria
  - B: Total Plate counts
  - QC: temperature control (quality control)

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Results summary

				sample A		sample B	
Lab ID	Delivery date	Date of analysis	Temp. at arrival	E. coli 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

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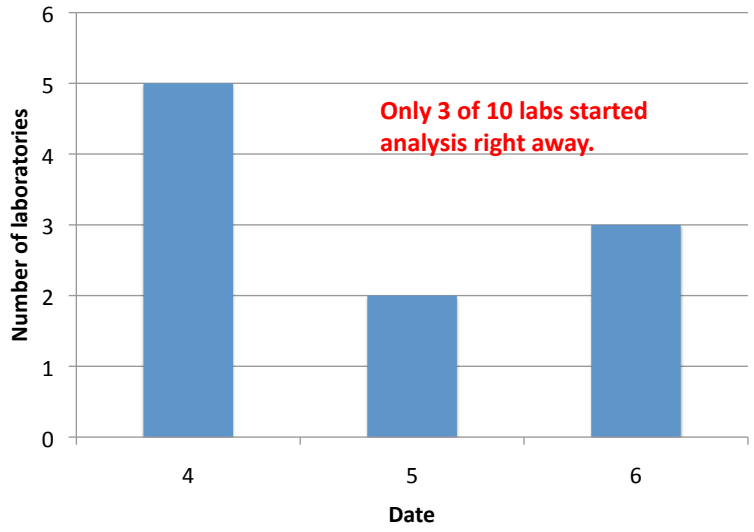
Delivery times



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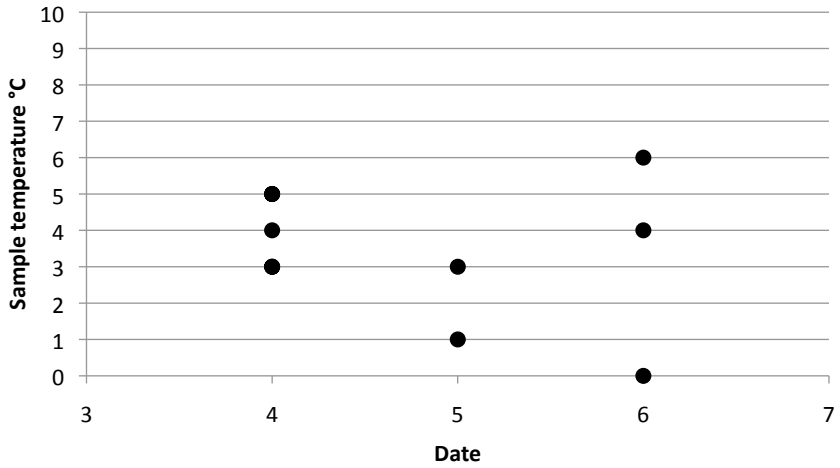
Temperatures at delivery



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Sample A: E. coli/Coliform bacteria

- Strain used: *Enterobacter aerogenes* NC no 10006; no E. coli ➔ All reported results correct

Lab ID	Delivery date	Date of analysis	Temp. at arrival	sample A		sample B	
				E. coli CFU/100 ml	Coliform bacteria CFU/100 ml	TPC CFU/ml temperature 1	TPC CFU/ml temperature2
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

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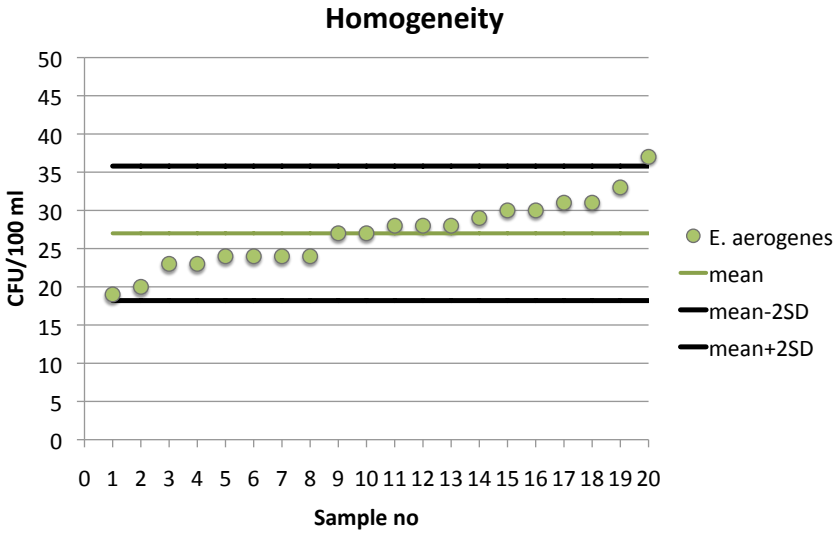
Sample A: E. coli/Coliform bacteria



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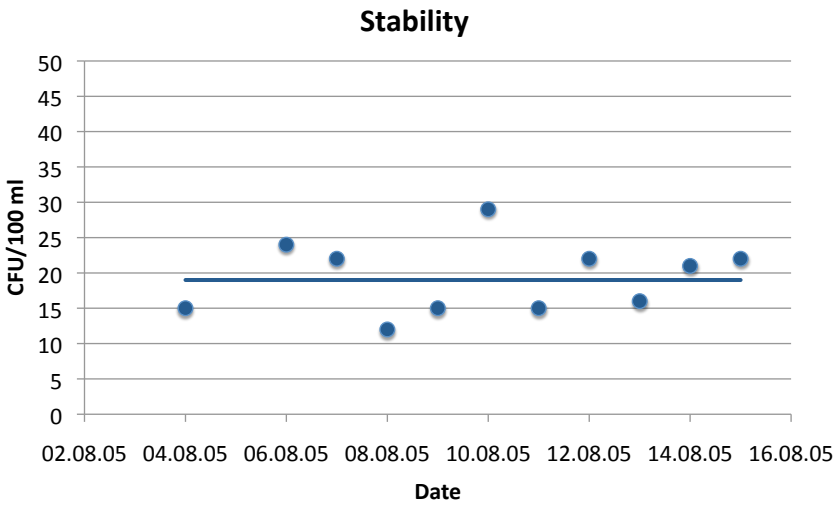
Sample A: E. coli/Coliform bacteria



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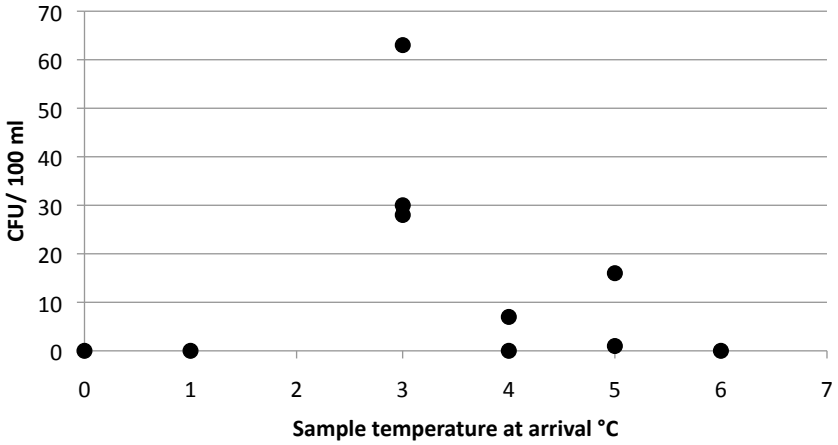
Temperature dependence of test results: Coliform bacteria



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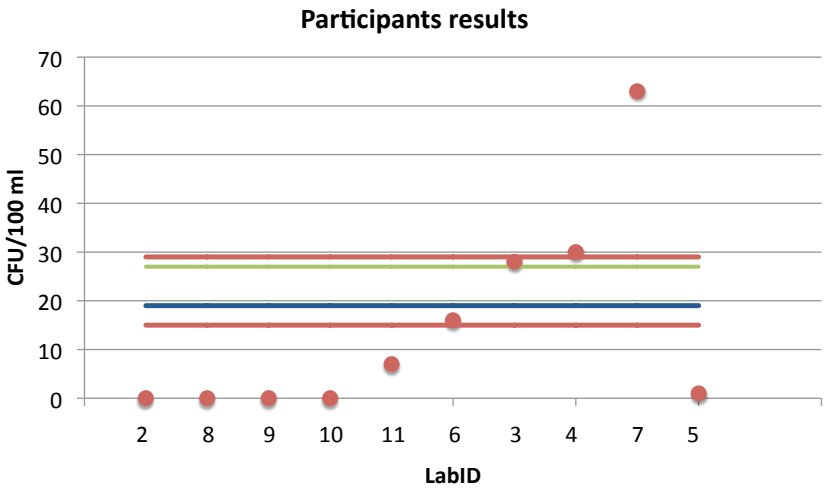
Sample A: E. coli/Coliform bacteria



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Sample A: E. coli/Coliform bacteria

- Target value?
  - Mean of homogeneity testing: 27 CFU/100 ml
  - Mean of stability testing: 19 CFU/100 ml
  - Mean of participants: all 15 CFU/100 ml
  - Mean of participants: pos. 29 CFU/100 ml
  - Algorythm A (ISO 13528)

Assigned value (Chemistry) ISO/IEC Guide 43-1:1997

- 5 ways to determine an assigned value (informative Annex)

- **Know value** – with results determined by specific test items
- **Certified reference values** – as determined by definite methods (for quantitative tests)

Assigned value (ISO Guide 43-1)

- **reference values** – as determined by analysis, measurement or comparison of the test item alongside a reference material or standard, traceable to a national or international standard

Assigned value (ISO Guide 43-1)

- **Consensus values from expert laboratories** – el should have a demonstrable competence in the determination of the measurand, using validated methods known to be highly precise and accurate, and comparable to methods in general use. The laboratories may, in some situations, be Reference Laboratories

Assigned value

– **Consensus values from participant laboratories** – using statistics... (no details given) with consideration of the effects of etreme values

ISO 13528:2005

- Generally same methods as in ISO guide 43-1
  - Preferred statistical method for consensus mean and standard deviation: Algorithm A

Sample B: Total Plate Count

- Strain used: *Enterobacter aerogenes*

LabID	CFU/ml
2	150
3	12
4	13
5	45
6	32
7	>300
8	6
9	10
10	4
11	0

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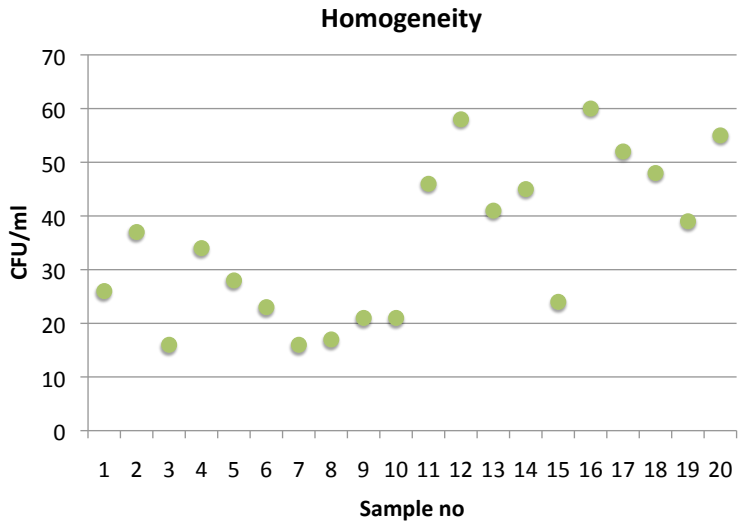
Sample B: Total Plate Count



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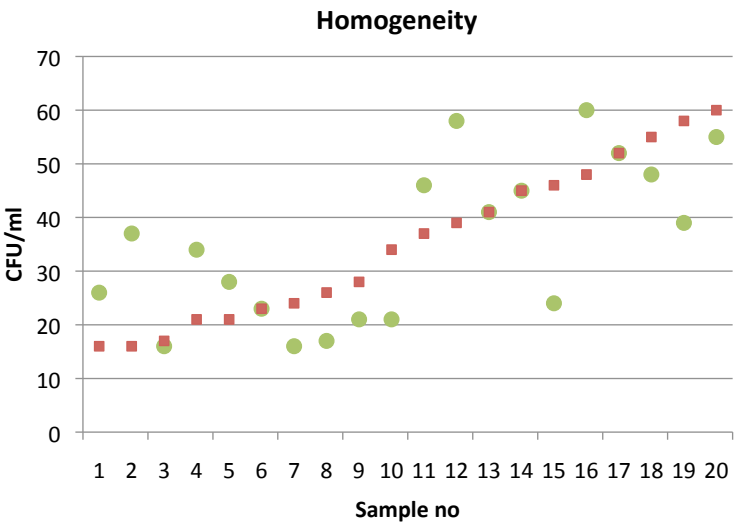
Sample B: Total Plate Count



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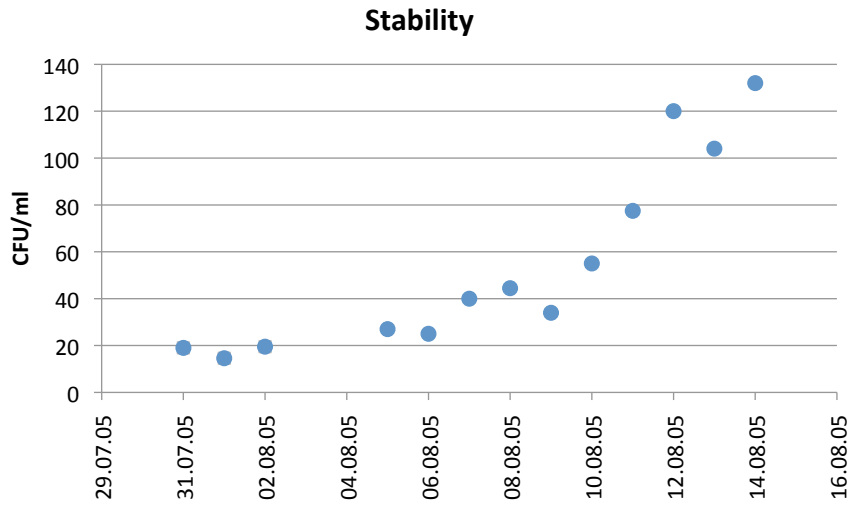
Sample B: Total Plate Count



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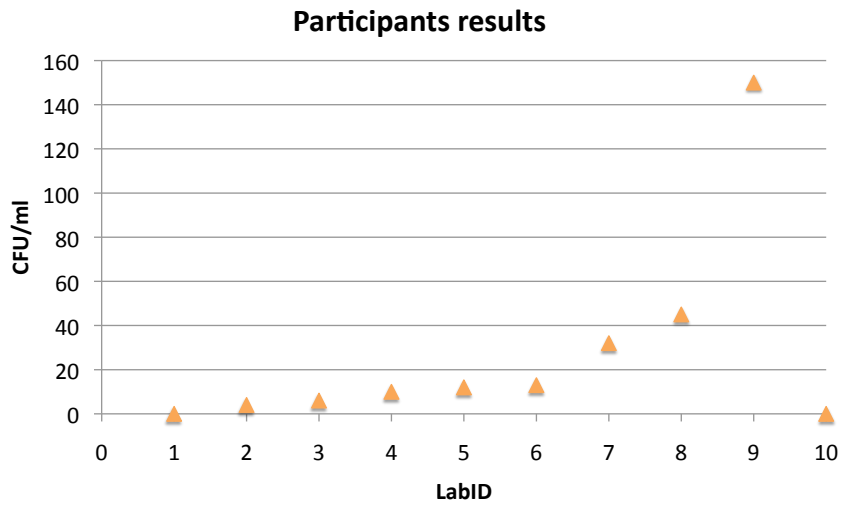
Sample B: Total Plate Count



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Sample B: Total Plate Count

- Homogeneity data and stability data look good but do not fit together
- Participants results do not match…
- How to approach corrective actions e.g. Plate count method

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Steps to keep under control in TPC

- Preparation of media (ready constituted media) – pH, expiry date, efficacy, sterility, constituants, 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

Steps to keep under control in TPC

- bring sample to room temperature
- Homogenizing of the sample
- Pipetting – calibration
- Sterilization of glassware (cool to room temperature)
- Labelling (no mix up of samples)
- Duplikates might be nessessary
- 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

Steps to keep under control in TPC

- 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

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Standards in use:E. coli/Coliforms

Method	Medium	Temp.	time
9308-1	Filter membrane		24 h
1. Presumptive 2. Pour plate	McConkey broth VREA	37°C 48 h 37°C 48 h	
9308-1	MLSB		44 +-0.5
9308-1	VRB	31°C	24 h
RS!5; RS217-3 KS220	VRB		48
Membrane filtration	VR		24
9308-1	VRBL	37	24
9308-1	LTTC	37	24
Membrane filtration	LSB	35	24
Spread Plate	MacConkey	37	48
7251		37	

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Standards in use: TPC

Temp1	Temp2	Method	Medium	Time (h)
30	37	Pour plate	PCA	48
37	37	Pour plate	Agar	48
37		ISO 8199; ISO 19458	SPCA	48
37	22	Pour plate	PCA	72 / 72
22	37	Pour plate	PCA	72
37		ISO 8199	PCA	48
37		4833	PCA	48
35		Pour plate	PCA	48
37		Spread plate	PCA	48
37		4833		

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Flyer

- Names of the local coordinators
- Membership and Waterlab association
- Rephrase first paragraph about water in the region (omit?)
- Mauritius adress is incorrect: Bell village delete It is Moka
- Coliform bacteria/E. coli
- Total plate count method
- How to join us?
- Add seychelles local coordinators
- Scientific consultant

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Benefits of 2nd Micro-PT

- What benefits did you have from of 2nd Micro-PT evaluation?
- Way forward (to do), improvements
- How can more participants be attracted?
-

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What benefits did you have from of 2nd Micro-PT evaluation?

- Networking
- Realize mistakes
- Ways to improve / improvement opportunities
- Pass the massage to manager how important PT is
- Assesses the status of competence of lab
- Gives chance to assess competence of staff
- Assesses test method capabilities
- Confidence building in own capabilities
- Confidence during audits

- Sample more stable/repeatability was OK
- Evaluation of reproducibility testing was possible

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Way forward?

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

Attract more participants through

- Meetings with local coordinator
- Separate local coordinator 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 point office
- Initiate formation or contact national lab associations for raising awareness

Examples of statistical approaches to value assignment in Chemistry

Your measure of excellence

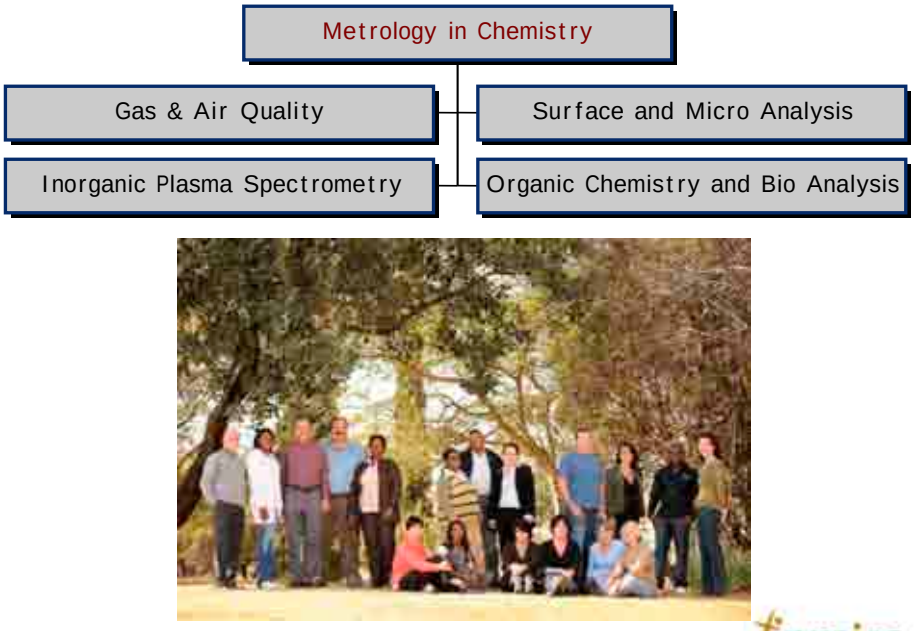
Introduction

- Background
  - CCQM
  - Chemistry at NMISA
- Statistical approaches to value assignment in Chemistry Interlaboratory studies
- Examples from CCQM Intercomparison Studies

Background: CCQM

- Metrology in Chemistry: Science of Measurement in Chemistry
- Responsibility to promote the concepts of:
  - International traceability to SI
    - Amount of substance (mole)
    - Mass fraction (kilogram)
  - Reduce Technical Barriers to Trade
- Mutual Recognition Arrangement (MRA)
  - Calibration and Measurement Capabilities (CMCs)
- Peer reviewed Quality system
- Proven technical capability (successful participation in relevant interlaboratory comparison studies)

nmisa



nmisa

Gas Metrology

- Established 1998
- Calibration of breathalysers
- Preparation of primary gas reference mixtures (PRMs) in nitrogen (N_2) and air matrices by gravimetry
  - CO_2; CO; NO; NO_2; SO_2; H_2S; C_3H_8; Stack gas mixtures
- Purity analysis
  - GC-FID; GC-PDHID; FTIR; NDIR; GC-MSD; CRDS
- Certification of gas mixtures
- Calibration of air pollution analysers
- Accreditation: Gravimetric preparation of gas mixtures
  - ISO 17025
  - ISO Guide 34

Surface and Micro-analysis

- Established in 1998
- Instrumentation
  - XPS; SEM-EDS / EBSD; TOF-SIMS; XRD; GD-OES
- Focus Areas
  - Industrial support
    - Imaging (nano-scale and elemental mapping)
    - Elemental composition and binding energies
    - Crystal structure
    - Surface layers and coatings (thickness and composition)
    - Surface chemistry (catalysis, functional groups)
  - Polymer research and analysis
    - FTIR-TGA
  - Proficiency Testing
    - Electron microscopy magnification calibration
    - Elemental analysis by EDS

IPS Laboratory

- Established in 2000
- Instrumentation:
  - HR-ICPMS, Laser Ablation ICPMS, Axial ICP-OES
  - Focus Areas: Trace and ultra-trace analysis in Food & Environmental samples
  - CCQM Intercomparisons:
    - Food, environmental, metal and metal alloys and advanced materials
  - Collaboration on Certification of Reference Materials:
    - Minerals, food, environmental samples
  - Participation / value assignment in selected PT Schemes:
    - IAEA – AFRA: Nuclear Research Reactors and Analytical laboratories in Africa
      - Geological material / minerals
      - Food & environmental material
    - NMISA : Stainless steel – Elemental analysis by EDS
  - Support to Industry:
    - Maize, Animal supplements, Plastic, Nano-materials
  - Feasibility study: Primary Inorganic Standard Solutions
- Accreditation: ISO 17025

Organic Chemistry and Bio-Analysis

- Established 2001/2002
- Instrumentation:
  - GCxGC-FID; GC-MS; GCxGC-TOFMS; LC-MS; HPLC; DSC, UPLC/MS/MS
- Focus Areas:
  - Persistent Organic Pollutants (POPs)
  - Aqueous ethanol and sodium fluoride standards
    - Certified Reference Materials
    - Proficiency Testing Scheme: Department of Health
  - Mycotoxin analysis
  - Purity analysis on chemical compounds
  - Adulteration in foodstuffs and wine
  - Investigations into a bio-analysis capability
  - Method development for biodiesel analysis
- Accreditation: Preparation of aqueous ethanol and sodium fluoride calibration standards
  - ISO 17025
  - ISO Guide 34

Organic Chemistry and Bio-Analysis _ Microbiology

- No Microbiological facilities at NMISA
- BAWG: Bio-Analysis Working Group in CCQM
  - Currently no activity in the Microbiology area
  - At the last meeting the decision was taken to investigate further.
    - Will conduct a survey to ascertain international activities
    - Identify what could be attempted from the CCQM's side to support international comparability in microbiological analysis.
- Bio-Analysis representative has undertaken to distribute the relevant information to interested parties within Africa.

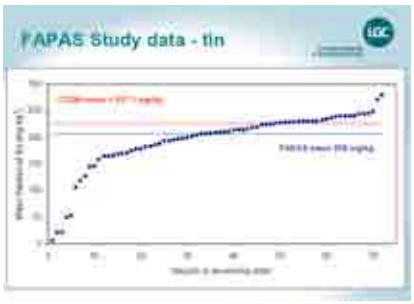
  NMISA: Desireé Prevoo

Proficiency Testing: Value Assignment in Chemistry (1)

- Independent reference value assignment
  - Gravimetric value
    - Accurate preparation and purity assessment required
    - Homogeneity and Stability could be an issue
  - Certified Reference value (CRM)
    - Expensive
    - Homogeneity and Stability assessed
    - Limited range of materials available
  - Independent Primary Method
    - SI-traceable reference value
    - Wide range of potential material available
    - Homogeneity and Stability could be an issue
  - Reference value (RM)
    - Longer traceability chain and larger measurement uncertainty, but SI-traceable reference value
    - Less expensive
    - Wide range of potential material available
    - Homogeneity and Stability could be an issue

Proficiency Testing: Value Assignment in Chemistry (2)

- Consensus value from expert laboratories
  - Careful selection and monitoring of expert laboratories
- Consensus values from participants
  - Most commonly used
    - Simple
    - Cost effective
  - Use of appropriate statistical tools
    - Estimation of the central location becomes critical, e.g. mean, median, weighted mean
    - Rejection of outliers, e.g. Dixon, Grubbs
  - Biased consensus values may be difficult to identify



CCQM Intercomparisons

- Reference Value = RV ± k·u(RV)
  - Gravimetric value (independent value)
  - Arithmetic Mean

$$X_{ref} = \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

    - Reject outliers only for technical reasons
  - Median

$$X_{ref} = \tilde{x} = \begin{cases} x_m & when\ n-odd \\ \frac{x_m + x_{m+1}}{2} & when\ n-even \end{cases}$$

    - Robust estimator - unaffected by extreme values or outliers
  - Weighted Mean

$$X_{ref} = \bar{x} = \frac{\sum_{i=1}^{n} \left(u_{x_i} \cdot x_i \right)}{\sum_{i=1}^{n} u_{x_i}}$$

    - Reject outliers only for technical reasons
    - Incorporates the uncertainties of the participants
  - Statistical software – incorporates the uncertainties of the participants
    - XGENLINE (NPL) – Regression functions
    - PDF-maker (NIST) – Mixture Model-Median
- Expanded Uncertainty associated with the Reference Value
  - According to the GUM (Guide to Expression of Uncertainty in Measurement)

Proficiency Testing: Statistical evaluation of performance

- Estimate of laboratory bias / Difference
  - $D_i = x_i - X_{ref}$
- Percentage difference
  - $\%D_i = \frac{(x_i - X_{ref})}{X_{ref}} \times 100$
- Ranks / Percentage ranks
  - Rank 1 = smallest difference from reference value
  - Rank p = largest difference from reference value
- z-score
  - $z = \frac{(x_i - X_{ref})}{\sigma}$
  - |z| > 2 : Warning limit
  - |z| > 3 : Action limit
- E_n-number
  - $E_n = \frac{(x_i - X_{ref})}{\sqrt{U^2(x_i) + U^2(X_{ref})}}$
  - |En| > 1 : Warning limit
  - Reliable estimation of uncertainty required

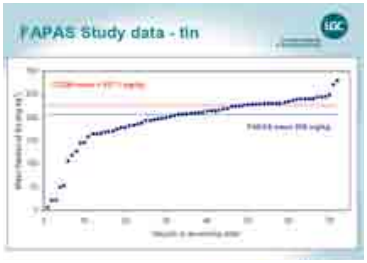
Proficiency Testing: Standard Deviation

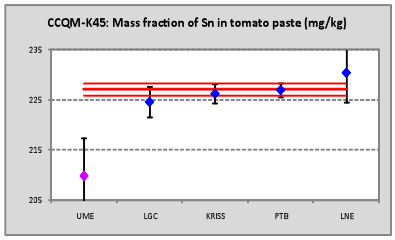
- Prescribed value
  - E.g. legislation
- General agreement amongst participants with regard to acceptable level
  - Fit-for-purpose
- Predicted by general statistical model
  - E.g. Horwitz-trumpet.
  - Prediction only based on concentration level, i.e. may not be appropriate for technique or sample material.
- Precision experiment
  - E.g. prescribed in standard method, if specific analytical method is prescribed
- Standard deviation of PT-results
  - May vary significantly between rounds based on participants' results
  - 5% of participants above warning limit

But, we are only going to look at examples of value assignment today…

Examples from CCQM Intercomparisons

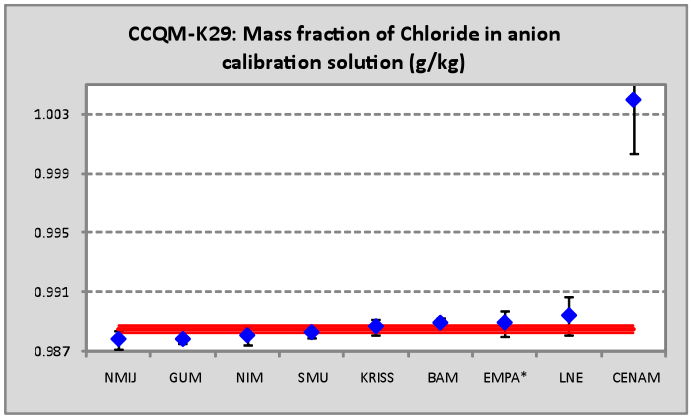
- Proficiency Testing
  - Participation open to all laboratories
  - Routine testing methods used
  - Large number of participants
  - Consensus value
- CCQM Intercomparisons
  - Participation restricted to NMIs, designated laboratories and selected expert laboratories
  - Best measurement method used
  - Often small number of participants
  - "Best estimate of the truth", i.e. SI traceable





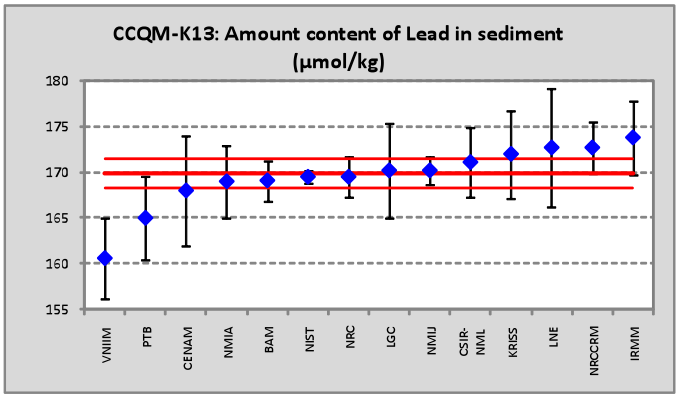
Examples: CCQM KCRV-estimation

- Gravimetric value: K29 (Cl^- in Calibration Solution)
  - The key comparison reference value is the gravimetric value x_{grav} of the Chloride mass fraction.



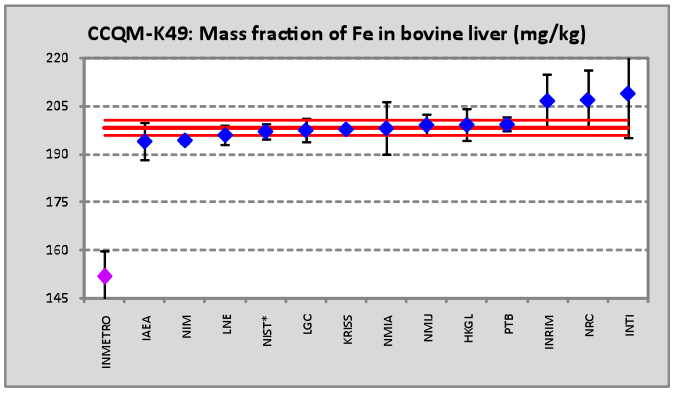
Examples: CCQM KCRV-estimation

- Normal distribution, large number of participating laboratories: K13 (Pb in Sediment)
  - The key comparison reference value, x_R, is calculated as the median of all results.



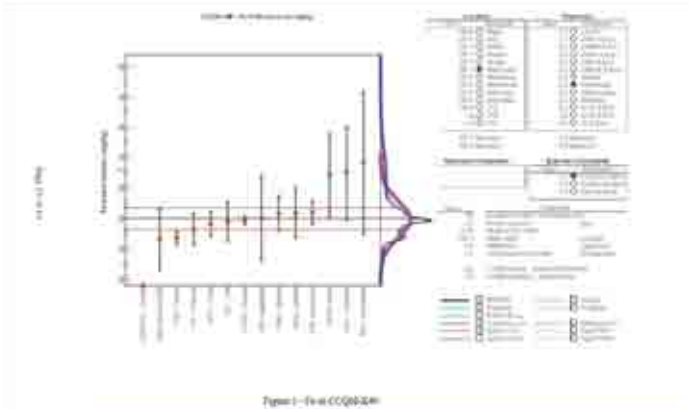
Examples: CCQM KCRV-estimation

- Normal distribution, large number of participating laboratories: K49 (Fe in Bovine Liver)
  - The key comparison reference value , x_R, is computed as the Mixture Model Median of the participant results excluding those from INMETRO.



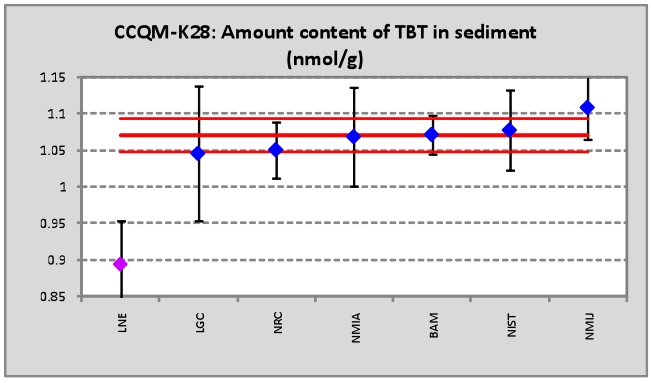
Examples: CCQM KCRV-estimation

- Normal distribution, large number of participating laboratories: K49 (Fe in Bovine Liver)
  - The key comparison reference value , x_R, is computed as the Mixture Model Median of the participant results excluding those from INMETRO.



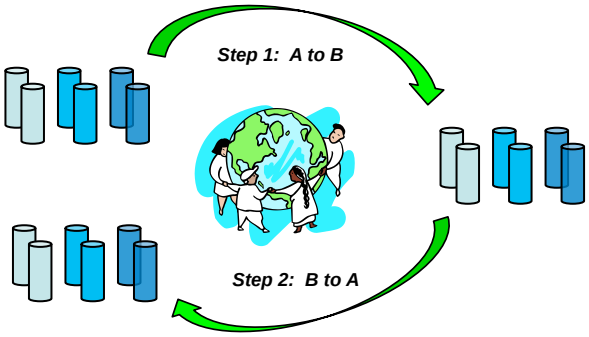
Examples: CCQM KCRV-estimation

- Outliers, small number of participating laboratories: K28 (Tributyltin in sediment)
  - The key comparison reference value, x_R, is calculated as the median of all results, except those obtained by the LNE.



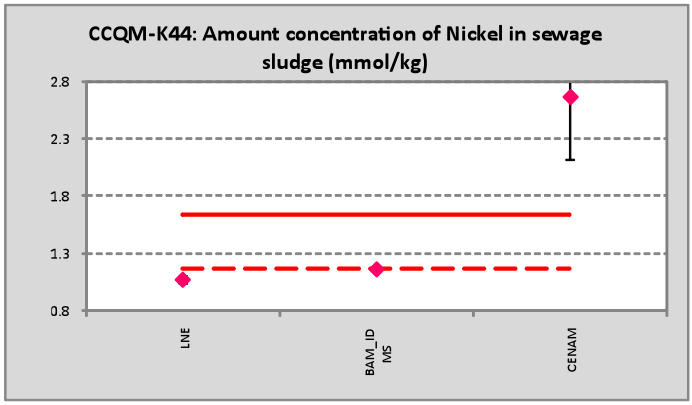
Examples: Identification of sources of problems in CCQM Studies

- CCQM-K73: "Round-trip" test conducted to monitor the effect of transport on samples
  - 0.01mol/kg HCl
  - Monitored mass change
  - Check stability of standard solution



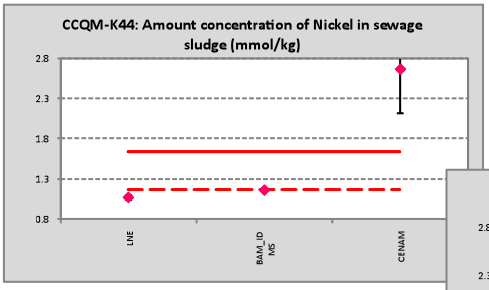
Examples: Identification of sources of problems in CCQM Studies

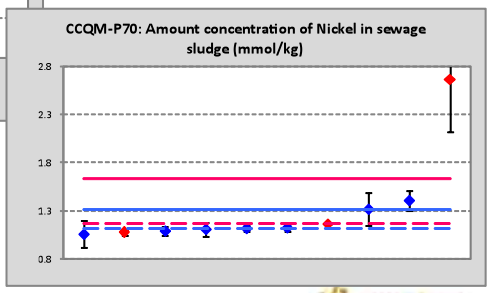
- No consensus, small number of laboratories participating: K44 (Ni in Sewage Sludge)
  - Large discrepancy between mean and median.



Examples: Identification of sources of problems in CCQM Studies

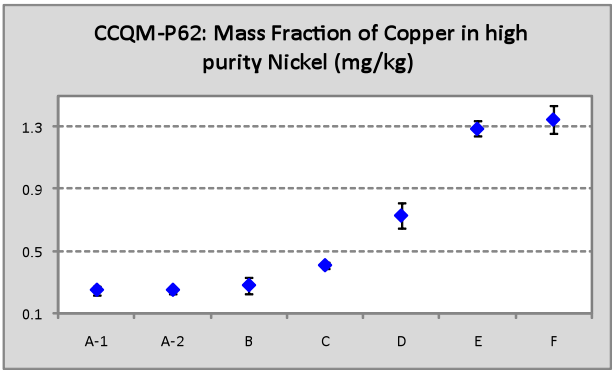
- No consensus, small number of laboratories participating: K44 & P70 (Ni in Sewage Sludge)
  - Obtain additional data. This supports use of median rather than mean.





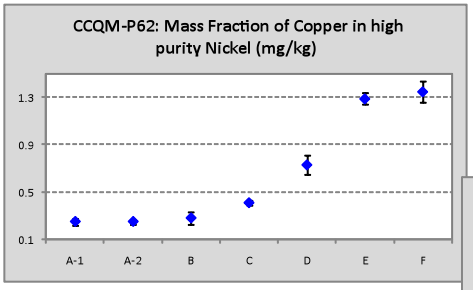
Examples: Identification of sources of problems in CCQM Studies

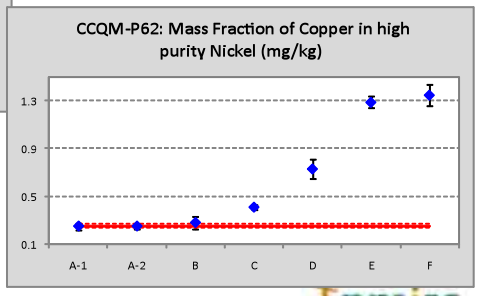
- No consensus, sufficient number of laboratories participating: P62 (metallic impurities in Nickel)



Examples: Identification of sources of problems in CCQM Studies

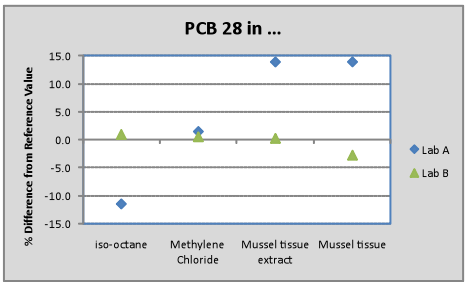
- No consensus, sufficient number of laboratories participating: P62 (metallic impurities in Nickel)
  - Primary method: ID-ICP-MS subsequently used to anchor results





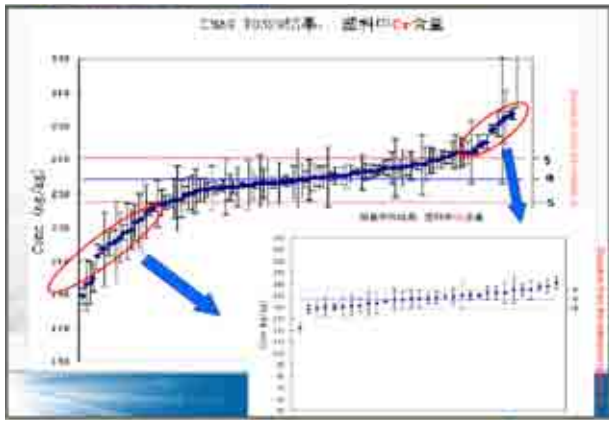
Examples: Identification of sources of problems in CCQM Studies

- Design a suite of studies: PCB congeners in various matrices to identify potential problems in calibration standards used, extraction methods used and instrumental analysis techniques used.
  - CCQM-K40 & P31.b.1:
    - PCBs in *iso*-octane
  - CCQM-P57:
    - PCBs in methylene chloride
    - Mussel extract in methylene chloride
  - CCQM-P67:
    - PCBs in Mussel tissue



Food for thought:

- **Example from National PT Study in China**
- **Corrective Actions implemented**



Conclusions

- There is no single solution. Evaluate on a case-by-case basis (employing statistically sound estimators)
- Always investigate potential technical explanations. Do not reject on statistical grounds alone.
- Insufficient data
  - No conclusion
  - Further experimental work required
- Corrective Actions

Acknowledgements

- CCQM Intercomparison participants
- DTI, South Africa

Thank you

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NMISA: Chemical analysis in Food

Element	Matrix	Technique
Pb, Cd, Cu, Fe	Red Wine	Double-ID-ICPMS
Cu, Zn, Fe, Ca	Soybean powder	Double-ID-ICPMS
Pb	Maize powder	Double-ID-ICPMS
Cd	Rice flour	Double-ID-ICPMS
Sn, Pb	Tomato Paste	Double-ID-ICPMS
Se	Pharmaceutical supplement	Double-ID-CV-ICPMS
Fe, Zn, Pb, Cd	Bovine Liver	Double-ID-ICPMS

Analyte	Matrix	Technique
Veterinary drug residues - antibiotics e.g. chloramphenicol	Bovine Milk, Pork muscle	IDMS HPLC/MS/MS
Pesticides, polychlorinated biphenyls	Mussel tissue	GC-MSD, GCxGC-TOFMS
Selenomethionine	Wheat flour	IDMS UPLC/MS/MS
Mycotoxins Aflatoxins Fumonisins Ochratoxin	Maize, grains, nuts, wine, milk	IDMS UPLC/MS/MS
Nutrients Fat soluble vitamins Water soluble vitamins	Infant formula, infant cereals	IDMS UPLC/MS/MS

TC 147/SC 4 - Microbiological methods

Items to be displayed:

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

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

Standard and/or project		ICS
ISO 6222:1999 Water quality -- Enumeration of culturable micro-organisms -- Colony count by inoculation in a nutrient agar culture medium	90.20	07.100.20
ISO 6340:1995 Water quality -- Detection and enumeration of Salmonella	90.92	07.100.20
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	07.100.20
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	07.100.20
ISO 7704:1985 Water quality -- Evaluation of membrane filters used for microbiological analyses	90.93	07.100.20
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	07.100.20
ISO 7899-1:1998/Cor 1:2000	60.60	07.100.20
ISO 7899-2:2000 Water quality -- Detection and enumeration of intestinal enterococci -- Part 2: Membrane filtration method	90.93	07.100.20
ISO 8199:2005 Water quality -- General guidance on the enumeration of micro-organisms by culture	90.93	07.100.20
ISO 9308-1:2000 Water quality -- Detection and enumeration of Escherichia coli and coliform bacteria -- Part 1: Membrane filtration method	90.93	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/WD 9308-2 Water quality -- Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli -- Part 2: Multiple tube (most probable number) method	20.20	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	90.20	07.100.20

count media used in water quality tests		
ISO 10705-1:1995 Water quality -- Detection and enumeration of bacteriophages -- Part 1: Enumeration of F-specific RNA bacteriophages	90.93	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	90.60	07.100.20
ISO 10705-4:2001 Water quality -- Detection and enumeration of bacteriophages -- Part 4: Enumeration of bacteriophages infecting Bacteroides fragilis	90.93	07.100.20
ISO 11731:1998 Water quality -- Detection and enumeration of Legionella	90.93	07.100.20
ISO 11731-2:2004 Water quality -- Detection and enumeration of Legionella -- Part 2: Direct membrane filtration method for waters with low bacterial counts	90.93	07.100.20
ISO/WD 12869 Water quality -- Detection and quantification of Legionella and/or Legionella pneumophila by concentration and genic amplification by polymerase chain reaction (RT-PCR)	20.20	07.100.20
ISO/TR 13843:2000 Water quality -- Guidance on validation of microbiological methods	60.60	07.100.20
ISO 15553:2006 Water quality -- Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water	90.20	07.100.20
ISO 16266:2006 Water quality -- Detection and enumeration of Pseudomonas aeruginosa -- Method by membrane filtration	90.20	13.060.70
ISO 17994:2004 Water quality -- Criteria for establishing equivalence between microbiological methods	90.93	07.100.20 13.060.70
ISO 17995:2005 Water quality -- Detection and enumeration of thermotolerant Campylobacter species	90.93	07.100.20
ISO/DIS 19250 Water quality -- Determination of Salmonella species	40.60	07.100.20
ISO 19458:2006 Water quality -- Sampling for microbiological analysis	90.20	13.060.45
ISO/CD 29201 Water quality - The variability of test results and the uncertainty of measurement of microbiological enumeration methods	30.60	13.060.70

Evaluation questionnaire - Microbiology workshop

For evaluation of the workshops success please answer the following questions

How do you judge	Very good	Good	Fair	Poor	Very poor
The venue of the workshop	☐	☐	☐	☐	☐
The hotel (accomodation)	☐	☐	☐	☐	☐

How do you judge the different parts of the workshop?	Very useful 1	2	3	4	Not useful 5
The evaluation of the PT	☐	☐	☐	☐	☐
The working group discussion on TPC	☐	☐	☐	☐	☐
The troubleschooting session (TPC Checklist)	☐	☐	☐	☐	☐
The presentation on assigned values (NMISA)	☐	☐	☐	☐	☐
SADCWaterlab General assembly	☐	☐	☐	☐	☐

	Yes	No	Partially
Did the workshop fullfil your expectations?	☐	☐	☐

If no or partially please explain:

What were the most important topics to you?

What benefits did you draw from the workshop?