3rd Microbiology PT Evaluation Workshop within the SADCMET Proficiency Testing Scheme for Water Testing Laboratories

Report on the 3rd Microbiology PT Evaluation Workshop within the SADCMET Proficiency Testing Scheme for Water Testing Laboratories

Windhoek, Namibia, 1 - 4 November 2010

Prepared by Dr. rer. nat. Katrin Luden (scientific consultant)

Summary

This report summarizes the outcome of the evaluation workshop of the third Microbiology proficiency testing round. In August 2010 Uganda National Bureau of standards (UNBS) again provided microbiological test samples for proficiency testing of water laboratories. 33 laboratories from 16 countries participated in this PT round. Participants from 15 countries attended the workshop.

The evaluation of the PT round was started by hearing a report of the PT provider Jacqueline Kwesiga of UNBS. There had been problems with email communication and packaging. Both problems were addressed and future handling of the matters was discussed. The quality of the preparation was overall satisfactory. Logistics still poses challenges and will have to be improved.

Unfortunately evaluation of the reported data again showed that there was no consensus value between the participants results. There are no stable reference materials in water microbiology available resembling real water samples. Therefore the problem of how to come up with an acceptable reference value for the PT was addressed and discussed extensively. It was agreed that the Niedersächsisches Landesgesundheitsamt (NLGA, Germany, Dr. Luden) and two other laboratories that still need to be acquired should be used as expert laboratories. At least one of these should preferably be within the region.

Some laboratories reported results matching the quality control data of UNBS. These results can most probably be considered satisfactory. On the other hand a large portion of the information given with the results showed a strong need for improvement of laboratory procedures. The majority of the laboratories did not start analysis of the samples at the day of delivery. Considering the limited stability and purpose of the sample analysis needs to be started without delay.

In microbiology the method used influences very much the outcome of the analysis because the measurand often is defined by the method. It is therefore highly recommended to use internationally accepted methods e.g. ISO methods to produce comparable results. Working group discussions were used to compare the definitions of E. coli in the different methods used by the participants of the PT. As a result the workshop participants decided to write a recommendation on what methods are considered most suitable for their purpose of drinking water analysis.

All participants used the workshop to share experience with others and start networking.

Introduction

The workshop served to discuss the evaluation of the third microbiology proficiency testing round (potable water) provided in August 2010 by Uganda National Bureau of standards in the SADC and EAC region. It was held in conjunction with the evaluation workshop of the Chemistry PT provided by Namwater, Namibia.

During previous workshops the SADCWaterLab Association had been formed to facilitate networking among water laboratories and a general assembly was held during the workshop.

Workshop

The workshop was attended by representatives of laboratories from Botswana, Burundi, Congo, Ethiopia, Ghana, Kenya, Lesotho, Mauritius, Namibia, Rwanda, Seychelles, Swaziland, Uganda, Zambia and Zimbabwe. All participants and the german experts were welcomed by Namwater representative Dr. Shivute (CEO) the SADCMET regional coordinator Mr. Masuku the PTB representative Ms Wunderlich and SADC WaterLab Association Chair Mrs. Mwambo. After the opening ceremony the workshop split into two groups chemistry and microbiology to evaluate the respective PT schemes.

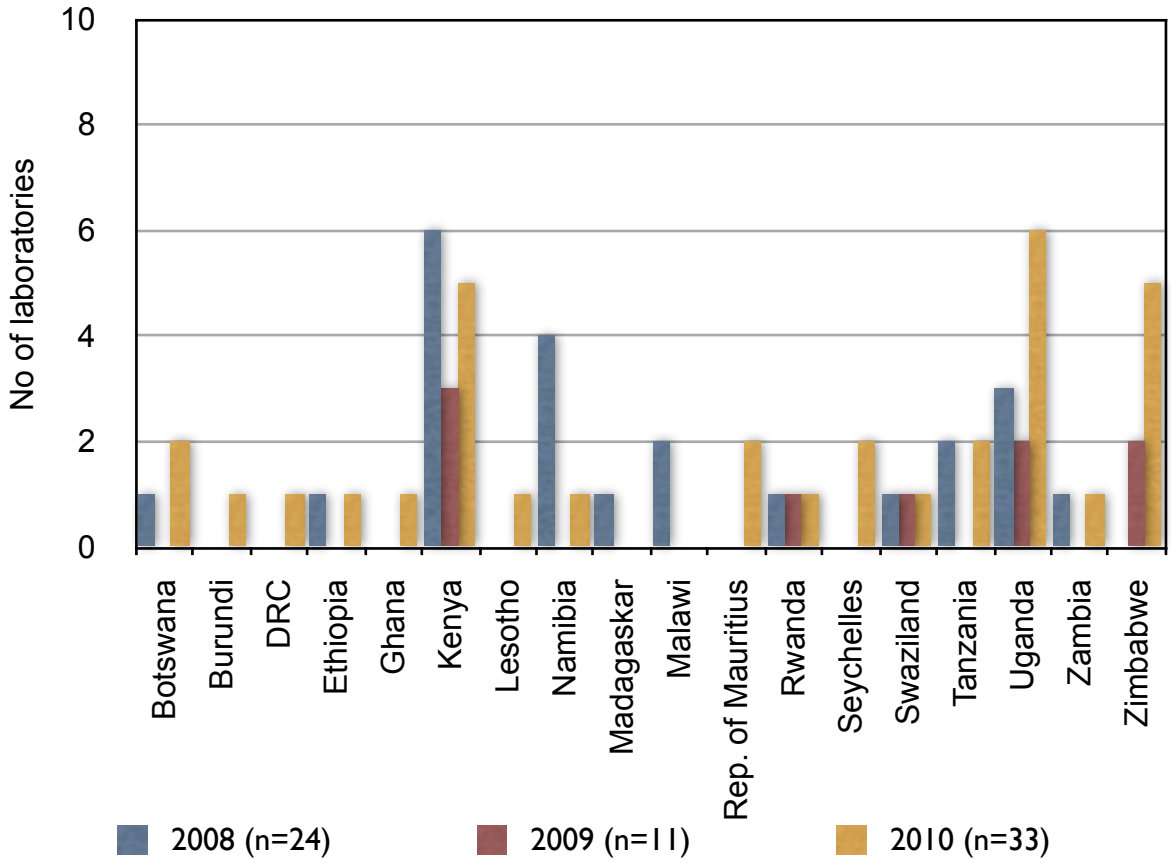


Figure 1: Participation in the microbiology PT 2008-2010

On this first day of the workshop the main focus was on the evaluation of the PT provider performance.

Report of the PT provider

The PT provider Jacqueline Kwesiga from UNBS reported how she had attracted a substantially larger number of laboratories to participate in the scheme than the previous year amounting in 33 participating laboratories in 2010. Two notifications were published using the mailing list arising from the two previous PT rounds and workshops, local coordinators, national accreditation focal points and the SADCMET website. Unfortunately correspondence by email did not work satisfactorily mainly due to unsatisfactory performance of hardware and server-provider. This has been addressed by installing a new server and contracting a new internet service provider. So hopefully communication will not be a problem in 2011.

Mrs Kwesiga described trial runs and several packaging simulations that were conducted in preparation for the 3rd PT. DHL was used as a courier as previously. Two different kinds of packaging had been used to distribute the samples. Samples were dispatched on the announced date and mostly received within a few workdays. Unfortunately some of the samples were frozen when arriving at the participating laboratories. This might be due to the very cold temperatures the ice bricks had been stored at before use in packaging. The packaging will have to be further improved. The only packages held up in customs were the ones to Mauritius. Some samples were reported to have reached the participants laboratory frozen. This shows that logistics for these samples closely resembling real potable water remains a challenge. It was agreed that a new packaging design should be prepared and tested before the next PT round.

The full presentation is given in Annex 1.

Evaluation

This proficiency testing scheme provided by UNBS is supposed to help assess the performance of laboratories in comparison with each other. It is therefore necessary to take precautions that this can actually be done. Taking into account the delicate nature of microbiological samples in terms of limited stability every participating laboratory should ensure that samples can be analyzed on the day of delivery. Unlike a routine sample that might reach the laboratory on the day of sampling transport of the PT sample takes at least one day due to the long distance it has to travel. It should be good practice for customer samples as well as for PT samples to be able to arrange for immediate start of analysis when needed. Only about half of the participants had initiated analysis right away. In four cases the reported day of analysis was even more than one day after delivery.

In order to enable all participating laboratories to arrange for immediate analysis upon arrival of the sample the PT provider will provide a copy of all accompanying letters together with a tracking number of the courier by email.

E. coli / coliform bacteria (Sample A)

According to UNBS quality control analysis of twelve randomly picked bottles of „sample A“ the mean bacterial concentration was approximately 36 CFU/100 ml. *E. coli* strain NC no. 09001 from NCTC (serotype 01) was used. These results show that distribution of bacteria in the sample was satisfactory (figure 1).

As the same strain and medium have been used for sample A and sample B one would expect survival of the strain in both samples in the same manner. Therefore laboratories detecting bacteria in only one of the samples should check all aspects of their methods for possibilities of improvement.

Variation of participants results was too high again to reasonably calculate a consensus value in order to be used as an assigned value according to ISO 13528. As there was no data from a second „expert laboratory“ available there is no way of knowing whether the UNBS dataset is biased. Therefore it should also not be used to set the assigned value to judge participants performance by statistical means. It nevertheless gives a good approximation of the concentration range the participants should at least have detected.

In order to avoid this unsatisfactory situation it was proposed for the next PT round that expert laboratories will be acquired in order to have an independently determined assigned value for statistical analysis.

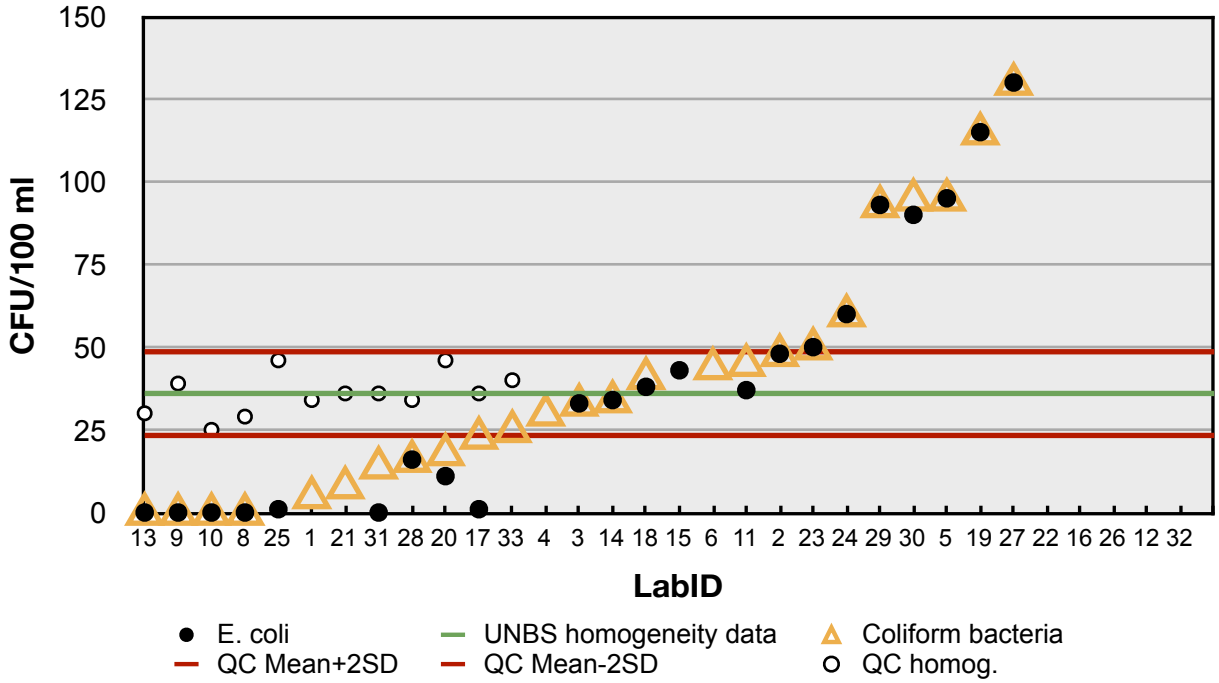


Figure 1: *E. coli*/Coliform bacteria sample A; Analysis of homogeneity and participants results. Homogeneity testing was done two days after dispatching the PT samples. 12 bottles of the original PT sample were analysed under repeatability conditions.

Total plate counts (Sample B)

According to UNBS quality control analysis of 15 randomly picked bottles of „sample B“ the mean bacterial concentration was approximately 79 CFU/ml. *E. coli* strain NC no. 09001 from NCTC (serotype 01) was used as in sample A. These results show that distribution of bacteria in the sample was satisfactory. Stability of the sample had been monitored for the whole week of shipment (7 days) and can at least be considered very good until Friday 7th of August.

Results reported to the PT provider varied largely and only very few results matched the UNBS data range. As most of the laboratories that reported results too high for sample B have reported reasonable results for sample A it is unlikely that this discrepancy is

solely due to growth during prolonged transport or warming of the sample. There is a checklist in the workshop report of the 2nd PT workshop (2009) dealing with all kinds of mistakes possibly connected to plate count analysis that can be used for help with corrective actions.

Detailed information on quality control data etc. Is given in Annex 2.

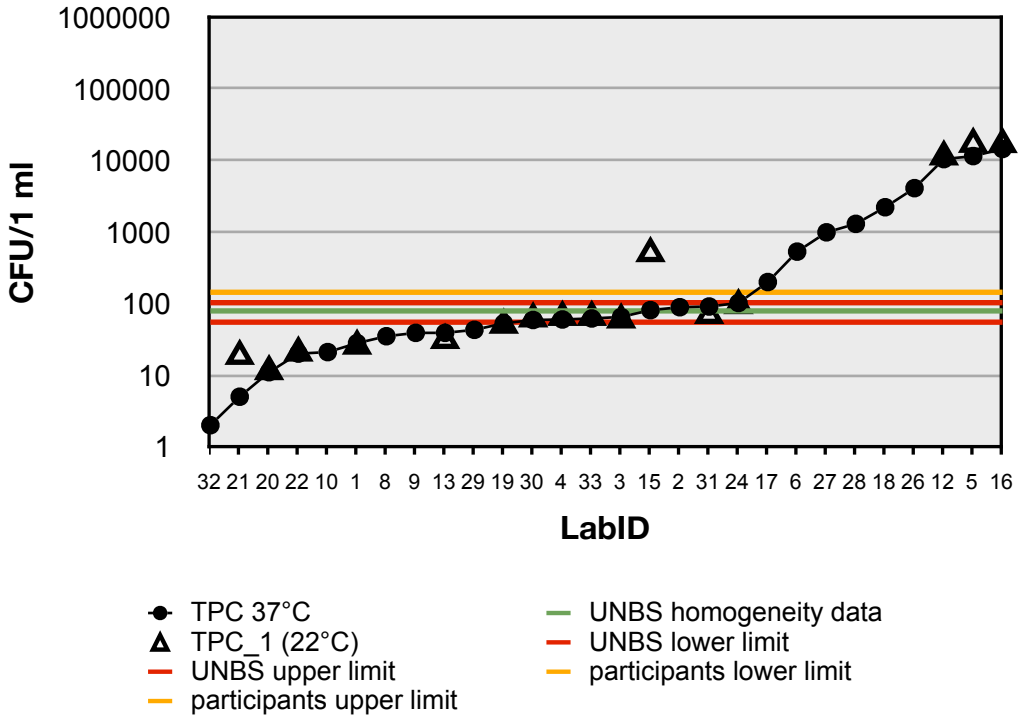


Figure 2: Total plate counts - Results of participants compared to quality control data (homogeneity testing) of UNBS.

Assigning target values

In order to objectively evaluate the performance of a laboratory within a proficiency testing scheme it is necessary to provide a reference or assigned value. The easiest and cheapest way of doing this would be to use the consensus mean of the participants results determined by robust statistics as described in ISO 13528. But again this was not possible for this PT round. As there are no traceable and stable reference materials available. The need to come up with an assigned value or reference value for evaluation of the PT was recognized by the participants. Dr. K. Luden shortly presented several possible ways that were afterwards discussed in working groups. It was agreed that UNBS and Dr. K. Luden will try to find laboratories suitable as expert laboratories. NLGA can serve as one of these and shipment of samples should be tested as soon as possible. There should be at least two more laboratories in this role preferably at least one within the south african region.

Full presentation see Annex 3.

Methods:

All participants had been asked to give detailed information on the methods used for analysis of the PT samples with their results. Sometimes an ISO standard was cited but the method described did not match the ISO standard.

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

Participants compared within small working groups the methods for E. coli/Coliforms used in different laboratories for PT analysis. Microbiological analysis of water samples is greatly operationally defined. E.g. methods for detection and enumeration of total coliforms use anything from lactose fermentation (gas and acid production from lactose) to enzyme activity (ß-galactosidase) to describe this group. It is not surprising that this leads to a very different set of species detected by various methods. Use of many different methods as used by the participants of the PT complicates and in the worst case prevents comparison of results within the PT scheme but also comparison of results from regular analysis in routine laboratory work. This problem was discussed in detail and a working group installed to come up with a recommendation on standardized methods best suitable for analysis of potable water in the SADC and EAC region. The recommendation is published on the SADCMET website the first SADCWaterLab Association Newsletter as well as given in Annex 4.

Training on method validation

Method validation was discussed using **ISO/TR 13843:2000 Water quality - Guidance on validation of microbiological methods**. The necessity to clearly define the purpose/scope of the method to be validated was stressed. Regulatory limits have to be taken into account as well as the matrix the method is supposed to be used with. For example a method for drinking water analysis of *E. coli* with a limit of 0 in 100 ml is not fit for purpose as long as a sample volume of less than 100 ml is used. After defining the purpose very clearly a basic description of target organisms and the method can be written. Experimental data has to be gathered from pure culture experiments as well as from natural samples. Numbers for sensitivity, selectivity, specificity, efficacy, rate of false positives and false negatives have to be calculated. Uncertainty of counts and the robustness of the method at the limits have to be checked. For quantitative methods it is also important to know the linearity range. To save a lot of work compared to a full scale method validation it is recommended to use an established standard (e.g. ISO) because this only has to be verified which adds to the benefit of comparability. (Presentation see Annex 5)

> *A method described in an established standard only has to be verified to prove that it works in the hand of the user according to its characteristic values (establishes that the method performs to its specifications).*

Training on measurement uncertainty

The topic of measurement uncertainty was shortly addressed during the workshop. Unfortunately there is no gold standard for calculating measurement uncertainty for microbiological methods. A few approaches were shortly introduced: the Top-down

approach (GUM/EURACHEM/CITAC) and the Bottom-up approach (VAM/NORDTEST) used in chemistry. Moreover there is the ISO CD 29201 as a standard under development that deals will the question of MU in microbiology. This ISO describes two ways the step-by step (GUM) and the global approach. (Presentation see Annex 6).

Conclusions and resolutions:

- Packaging should be done differently: the bottles need to be placed in sealed plastic bags that are to be labelled on the outside to prevent loss of labels from condensed water. Temperatures for freezing the ice bricks and packaging have to be tested again for optimizing the packaging procedure.
- For the next PT the provider will present an excel-sheet for reporting of the results in order to minimize transcription errors and give more space for information concerning methods used for analysis.
- The need to come up with an assigned value or reference value for evaluation of the PT was recognized. It was agreed that UNBS and Dr. K. Luden will try to find laboratories suitable as expert laboratories. NLGA can serve as one of these and shipment of samples should be tested as soon as possible. There should be at least two more laboratories in this role preferably one at least within the south african region.
- It was agreed that local coordinators and participants should go back to their countries and aggressively market the Microbiology PT scheme. The participants were particularly encouraged to make the local coordinators aware of this PT scheme. It was also acknowledged that while this task of marketing the scheme was the role of PT coordinators, these were in most instances Chemists. Hence it was proposed that these local coordinators could select Microbiologists who would hopefully do a better job.
- It was proposed that in future PT workshops emphasis should be given to methods and their critical control points or limitations/shortfalls - especially with regard to the recommended methods of analysis. This move was deemed to improve on the competence of laboratories particularly labs that had consistently performed poorly in the last 3 years. It was also deemed to be good for general learning purposes
- Future PT's could include training in sound Quality Management Systems particularly compliance to ISO 17025.

Laboratory visit at Namwater

The workshop was closed by a visit at the Namwater laboratory discussing some details of laboratory equipment.

Evaluation of the workshop by participants

An evaluation questionnaire was distributed for the workshop to be answered by the participants (Annex Q). All 14 participants handed back their questionnaires. The summary is given below.

How do you judge:	Very good 1	good 2	fair 3	poor 4	very poor 5	Mean
The venue of the workshop	7	7	0	0	0	1.5
The hotel (accomodation)	4	10	0	0	0	1.7
How do you judge the different parts of the workshop?	**Very useful**				**not useful**	
Report of the PT provider	5	4	3	2	0	2.1
Evaluation of the PT	8	5	1	0	0	1.5
Discussion on methods	6	6	2	0	0	1.7
Method validation	3	7	4	0	0	2.1
Measurement uncertainty	2	8	4	0	0	2.1
SADCWaterlab general assembly	4	6	3	0	0	1.9
Did the workshop fulfill your expectations?	11	0	2	0	0	1.3

Did the workshop fulfill your expectations? Yes/No/Partially If no or partially please explain. Answers Yes: 11 No: 0 Partially: 2

Explanation:

The validation and MoU was more of an overview. We would much appreciate a deeper inside of the subject of the matter.

I expected to get much more on how to validate methods and conduct the measurement of uncertainty.

What were the most important topics to you?	No of participants listing the topic
Method validation	9
Measurement uncertainty	7
Evaluation of PT results	7
Discussion on methods	6
Assigned values	2
Interaction/discussion of methods	1
Measurement uncertainty because some lab with poor results were required with assistance on how to improve	1
The discussed issues would help in addressing the technical challenges that we face in our laboratory and I expect improvement in our performance.	1
The breakdown of the results/rather the detailed discussion.	1
As a whole everything discussed during the 3 days were all important	1

What benefits did you draw from the workshop?
• From the workshop discussion I already know why we obtained unsatisfactory results for the PT. The workshop helped to better understand the requirement of methods and how to implement it.
• The workshop was also an opportunity to meet microbiologists from other countries and discuss various issues regarding microbiology that will surely help each other.
• As a first time participant I really got to know the benefits of the PT and the differences that are done at other labs which prompted me to do much more than I am doing now.
• I was able to learn a lot from experience of other laboratories which would in turn help me to improve on my methods and techniques and this is also vice versa for my colleagues.
• Gained more information (practical) which I can immediately apply in my lab.
• Method selection
• Method definition
• Method validation
• I have understood how a method used could bring about a variation in results Of course I have learned a lot apart from the PT evaluation.
• Its the recommendations done by the working group and the participants of the workshop on method comparison to help labs to adopt their report in a similar manner.
• I have learned a lot in method validation and measurement uncertainty and the challenges ...different labs and also possible routes of ... Them and assuring that the overall lab quality ... Is in place so as to ensure accurate reporting of the results consumer protection and facilitation of trade
• Personal discussion of PT results I better understand the influence of some factors on our PT results and would help address identified non-conformities
• One of the benefits was the discussion mede relating to the results obtained in relation to the method used
• Evaluation of results process was also well presented and beneficial
• Appreciation of what other labs are doing also came as a benefit.

Report prepared by Dr. rer. nat. Katrin Luden

Aurich 01.02.2011

SADCMET WATER PT EVALUATION WORKSHOP

MICROBIOLOGY WATER PT ROUND 3
WINDHOEK, NAMIBIA
1st – 4th November 2010

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

Background

- Trial Run 1 (25/05/2010): Used 2 organisms:

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

On the whole E.coli gave slightly higher counts than E.aerogenes. However both of these organisms were very stable.

- Trial Run 2 (24/06/2010): Used 1 organisms:

Enterobacter aerogenes NC no.1006 from NCTC .

Subjected a spiked drinking water sample to 2 temperatures 6^0C and 25^0C and analyzed portions at the aforesaid temperatures for 9 days.

Background

- Trial run 3 (17/07/2010): Tested the packaging with bottled water and ice bricks that had been stored at different temperatures -30^{0}C and -68^{0}C for 3 days in an environment maintained at 6^{0}C to simulate the cold chain of the courier.
- Readings from a temp. data logger showed that at the end of 24 hours there would be a lowering in temp. by 0.2^{o}C for packages which had ice packs stored at -68^{0}C as compared to those stored at 30^{0}C.

Background

Decided to use *Escherichia coli* NC no 09001 from NCTC (Serotype 01) for the following reasons:

- Wanted to have a change from the organism used last year and evaluate the performance of the above-named organism during 2010's PT round.
- To assess its performance with the courier (DHL). Since the courier maintained and had demonstrated from last years PT round that temperatures of ≤ 6^{0}C would be maintained throughout the distribution chain.

Preparation of 2nd Round

- **1st notification- 31st March 2010 – Closure date 1st May 2010.**
- **2nd notification (Remainder) – 10th May – Closure date 1st June 2010**
- Registration, Participation & return of results PT 2010: A total of 33 labs.

Significant improvement from last years performance. This year the number trebled - from 11 participating labs (2009) to 33 participating labs (2010).

Preparation of 3rd round - August

- Final Preparation of drinking water samples – 27/07/2010
- Bottling of samples - 1/08/2010
- Packaging of samples, pick up by the courier & dispatch of samples to various destinations – 2/08/2010

Preparation of 3rd round – Packaging/ Logistics

- Destination East Africa (Apart from DR Congo and Ethiopia)

  A slightly smaller Styrofoam package has been used. It contained 4 hard shell ice bricks that had been stored at the higher temperature of -30^{0}C for 72 hours prior to packaging. This is because delivery would take place within a day - 24 hours.

- Dimensions of Styrofoam packaging: 33 (l) X 19 (w) x 27 (h) cm

Packaging – East Africa

Preparation 3rd Round – Packaging/Logistics

- Other destinations:
  A slightly bigger Styrofoam package has been used. It contained 6 hard shell ice bricks that had been stored at the lower temperature of -68^{0}C for 72 hours prior to packaging. This is because delivery would take longer than 24 hours
- Dimensions of Styrofoam packaging: 35 (l) X 20 (w) x 40 (h) cm.
- The courier used was the same as last year – DHL and all the packaging was purchased from the courier.

Packaging outside East Africa including DR Congo and Ethiopia.

Preparation of 3rd round – Logistics/packaging

Accompanying envelope included:

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

Preparation of 3rd Round - Courier

- Courier advised that chilled temperatures (5^0C – 10^0C) were used on their cargo planes during transportation and in their cold rooms at the various ports.

Results of participants – Lab ID, Temperature of receipt, Date received, Date analysis started

Challenges and possible solutions courier/ Logistics

- 14 countries received their samples at temperatures >10^0C. 2 countries received at temperatures <0^0C. The ideal temperatures should have been 2^0C – 10^0C. The courier attributed the rise in temp. to transportation upon leaving the premises of the DHL office/ agent in the different countries right up to receipt in the laboratory$_1$

POSSIBLE SOLUTION.

- Participants to be provided with their airway bill numbers and ETA of packages on dispatch of packages.$_2$
- Courier has agreed to in future send out pre-shipment alerts to the various destinations urging their offices/agents to ensure samples are delivered in the shortest possible time and negate the effects of the outside weather.

Challenges and possible solutions – Courier / Logistics/ Packaging

- Mauritius had there samples impounded by Customs for over a week because Customs in Mauritius found the documentation presented to them unsatisfactory. The complaint was forwarded to the DHL office in Uganda and they are currently investigating it with DHL in Mauritius.

Challenges and possible solutions – Courier / Logistics/ Packaging

- Some of the participants did not receive the envelope that was packaged with the samples. The envelope contained a results sheet and a letter of instructions. Have been informed that this was most probably tampered with by the local customs and subsequently lost.

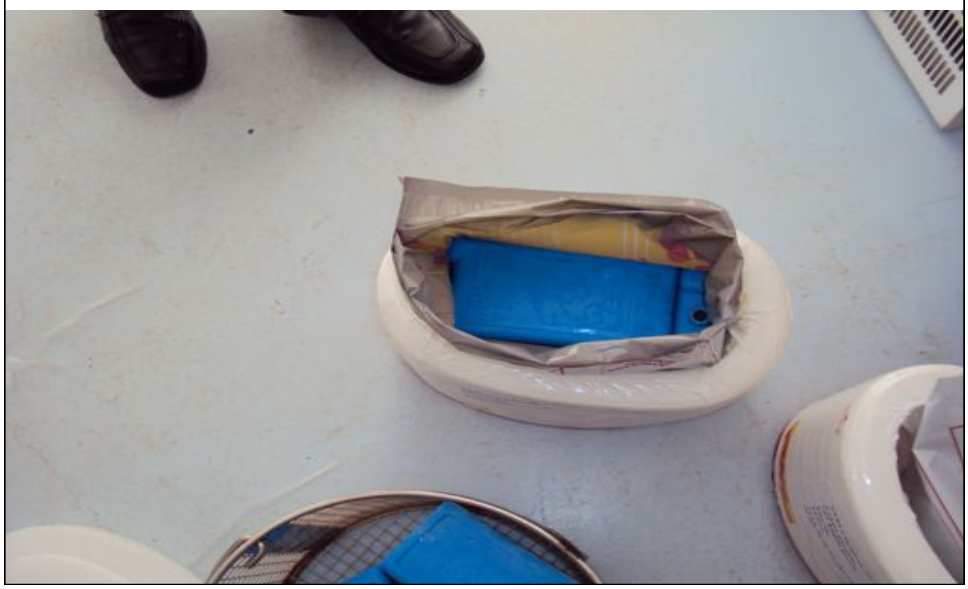
POSSIBLE SOLUTION

- Letter of Instructions and results sheets in addition to being sent in the samples will be emailed to participants at least one week prior to despatch of samples. This should prevent loss during transportation.

Challenges and possible solutions – Courier / Logistics/ Packaging

- Poor arrangement of plastic bottles and hard shell ice packs in the Styrofoam packaging

Challenges and possible solutions – Courier / Logistics/ Packaging



Challenges and possible solutions – Courier / Logistics/ Packaging

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How the arrangement might be improved

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Challenges and possible solutions – Courier / Logistics/ Packaging

- Another complaint from some of the participants was that the caps were not properly sealed

Communication Challenges and possible solutions

- Feed back from participants – Although the results sheet was designed to capture the required information, it was in some instances not conscientiously filled in by the participants.

POSSIBLE SOLUTION.

- This should improve with the dispatch of results sheets by email by the PT provider because participants will be able to type their data in making it more legible

Communication Challenges and possible solutions

- Some participants did not follow instructions as laid out in the PT notifications. They expressed willingness to participate but did not fill in a registration from and could not get samples sent to them because the delivery system is door to door unlike chemistry PT which is through local coordinators.

Communication Challenges and possible solutions

- The local Internet service provider used by UNBS caused a lot of problems with the use of Internet and email and at one point the server was malfunctioning.

POSSIBLE SOLUTION.

- A new Internet service provider has been contracted. Additionally a new server has since been procured and installed.

Final remarks

- A significant increase in terms of numbers of participation. Nevertheless more still needed to make a rigorous statistical analysis.

THANK YOU FOR LISTENING.

ANY QUESTIONS?

SADCMET Water PT
Evaluation Workshop
Microbiology Proficiency Testing
3rd Round

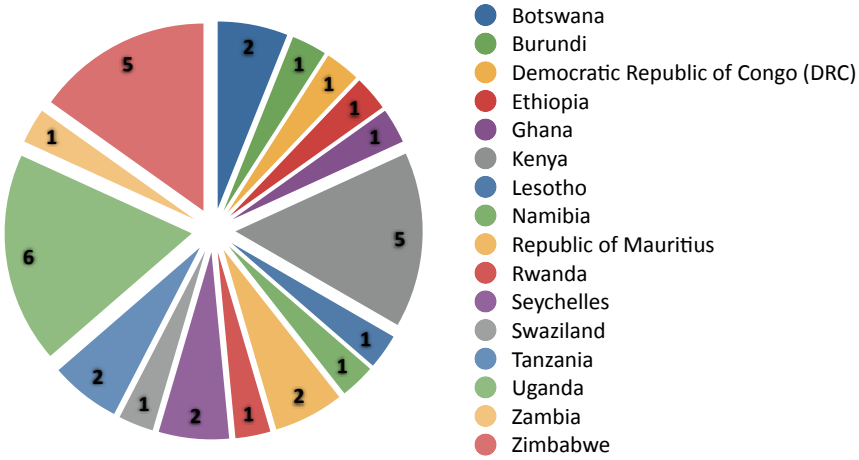
Schedule

- 1st Notification March 2010
- 2nd Notification May 2010
- Registration 01.05.2010
- Shipment 02.08.2010
- Deadline (extended) 14.08.2010
- Evaluation report 15.10.2010
- Workshop 01.-04.11.2010

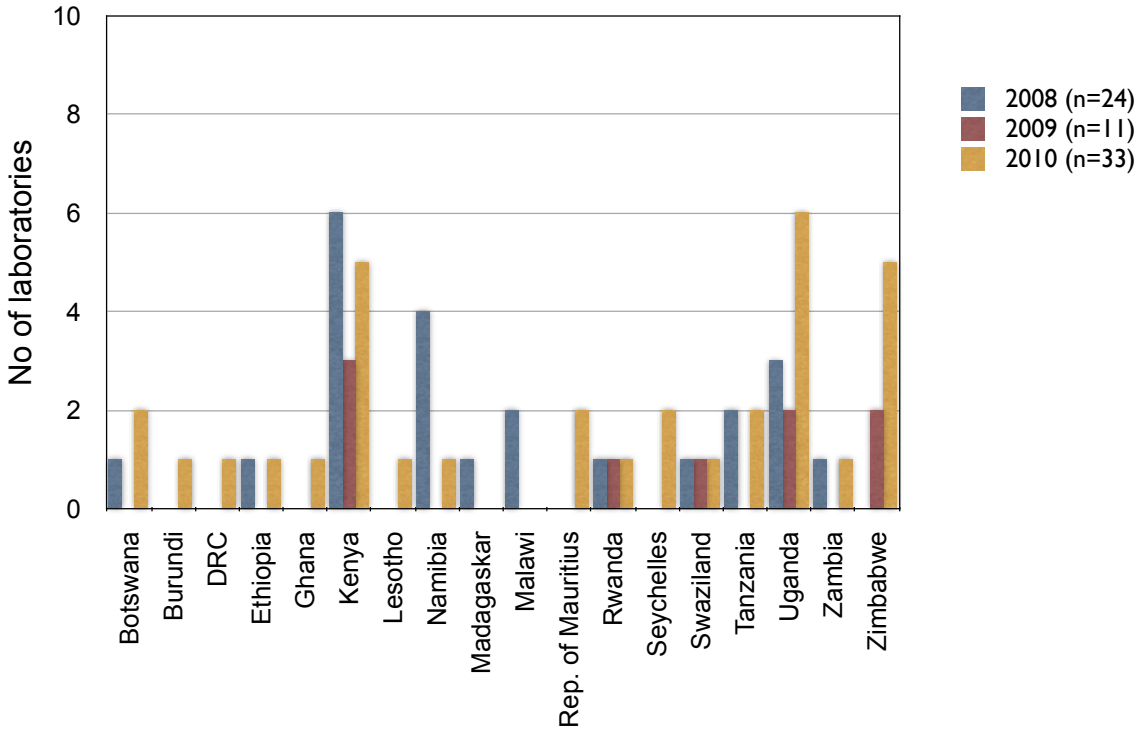
Principle of the PT scheme

- Liquid samples with living organisms
- Very realistic samples
- Limited Stability (7-10 days)
- Samples have to be stored at <10°C

Participation



PT development



General information

- Courier DHL
- 2 different packaging strategies
- 1 bottle for each parameter (*E. coli*/ Coliform bacteria and Total plate counts)
- 1 bottle for quality control (QC) for determination of temperature upon arrival at the laboratory

Evaluation part 1: Provider

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

Communication

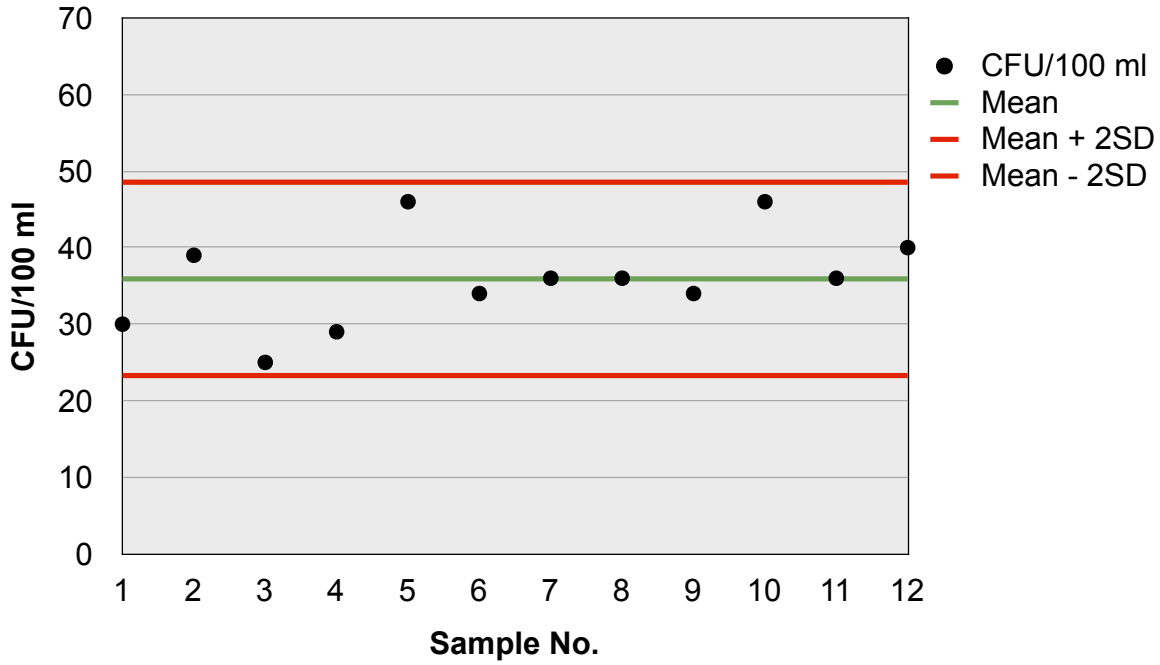
- At the 2009 workshop the next microbiology PT was scheduled for August 2010
- UNBS (J. Kwesiga) gave a first notification as scheduled in March
- 2nd notification in May
- Email was the preferred mode of communication
- Emails often did not reach UNBS
- Emails from UNBS never reached their destinations.
- Short term solution: use of private email-adress
- Long term solution .....

Preparation of samples

Quality control

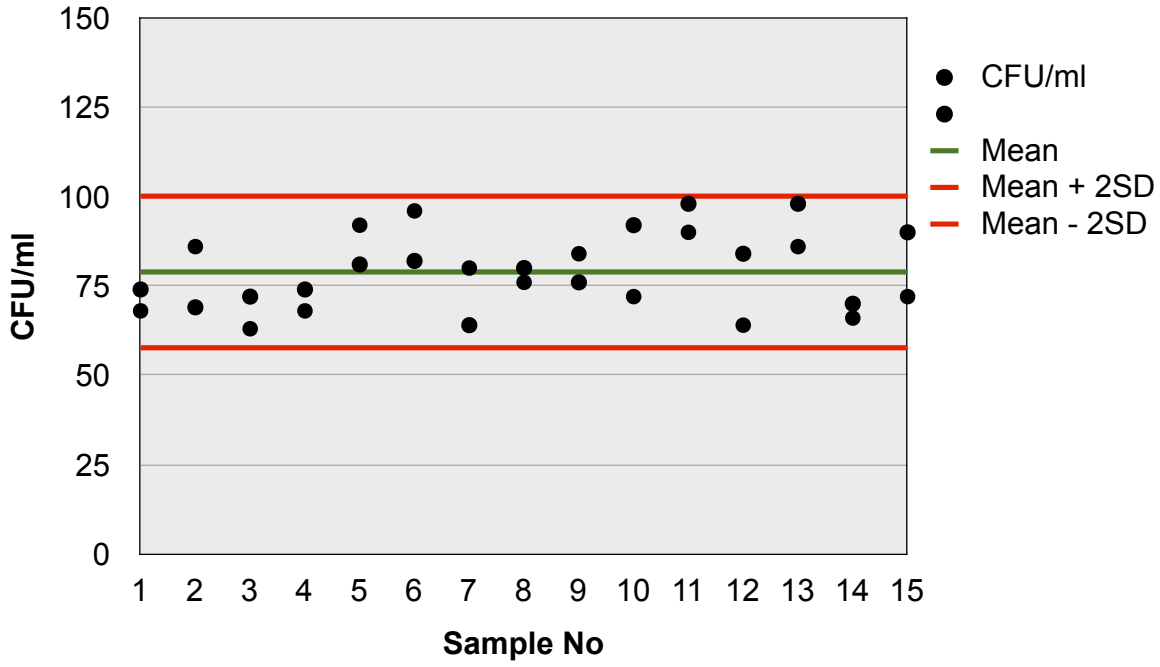
- Homogeneity Testing under repeatability conditions at UNBS on 05.08.2010 (day 3 after dispatch)
- Stability testing: analysis of 2 plates TPC with 1 ml each and 2 plates TPC with 0.1 ml each for 7 days after dispatch until 09.08.2010

Homogeneity sample A



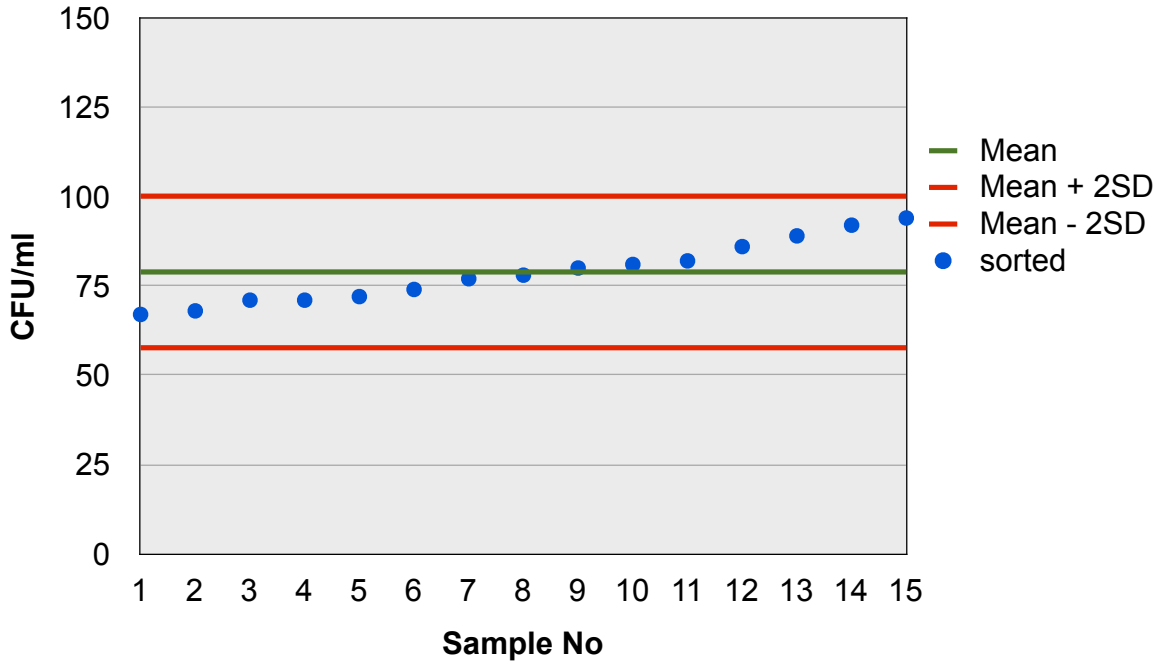
ISO 9308-1 (Membrane filtration, lactose TTC agar) was used for analysis on day 3 after dispatch

Homogeneity sample B



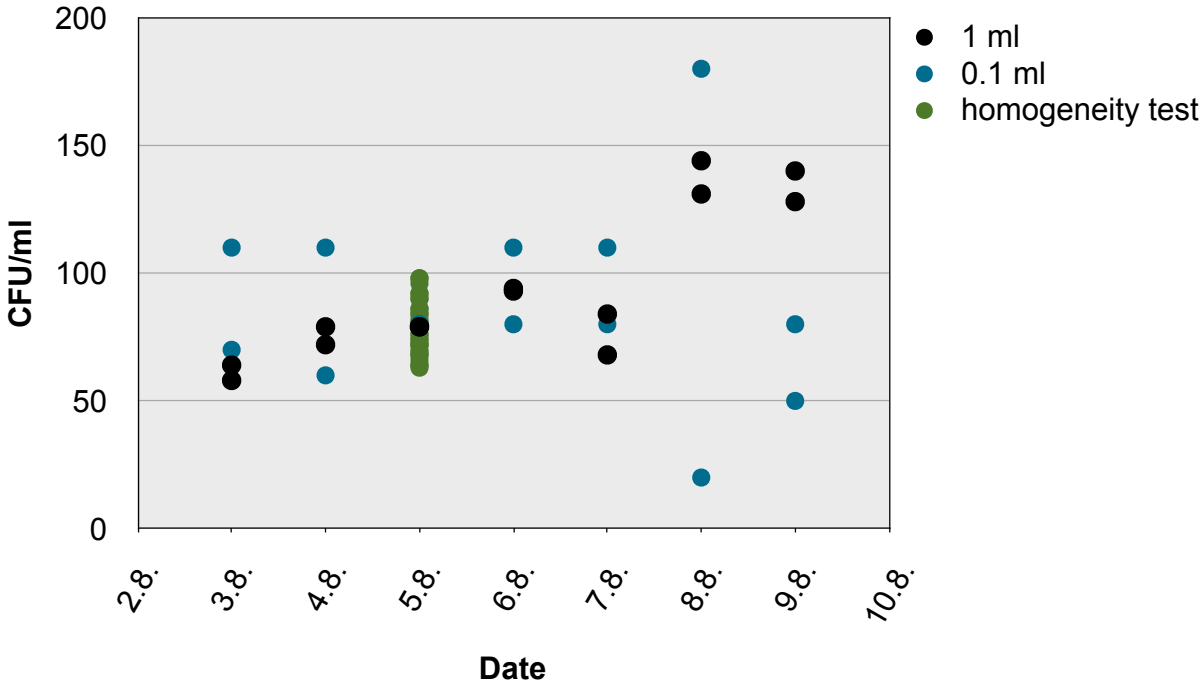
Total plate count method was used with two replicates per bottle

Homogeneity sample B



Total plate count method was used with two replicates per bottle

Stability sample B: TPC

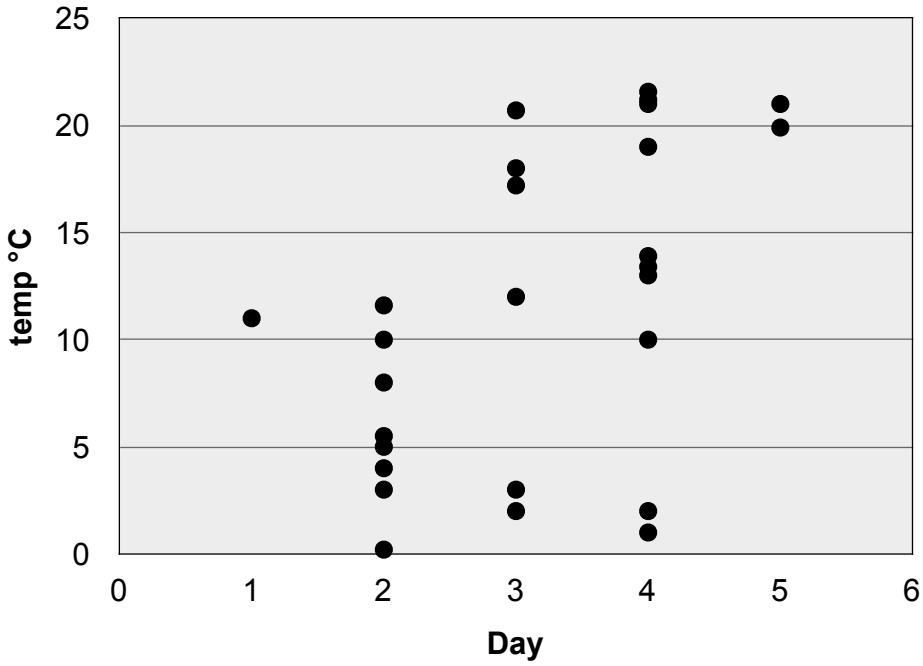


Preparation of samples

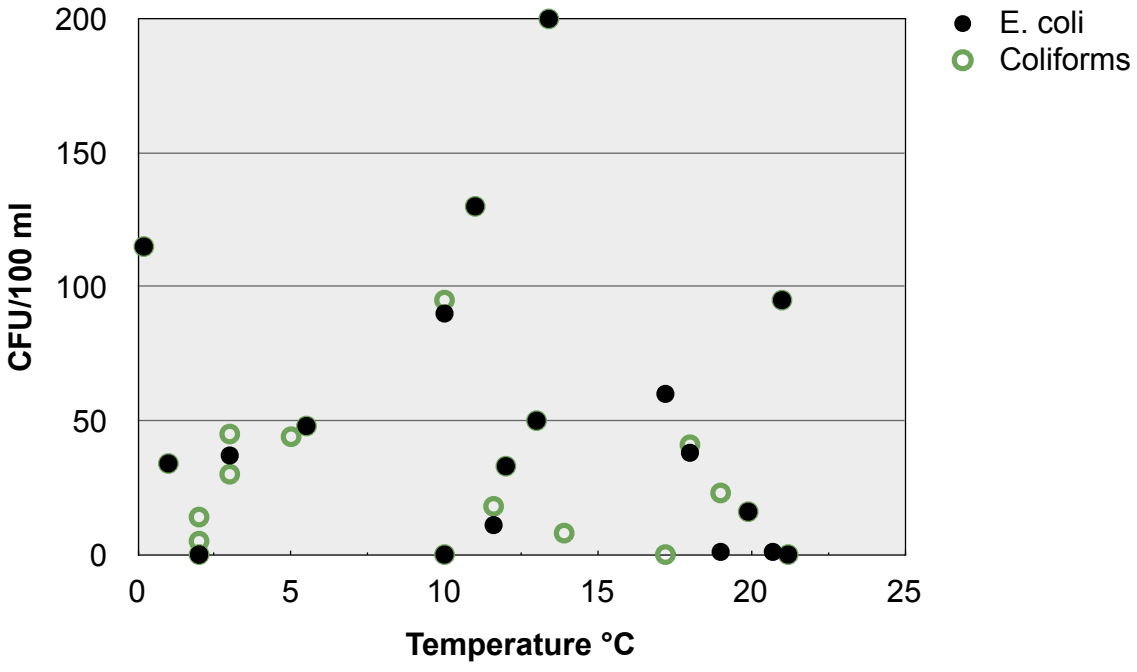
Quality control

- Homogeneity: colony counts of 12 (*E. coli*) and 15 (TPC) samples displayed random scattering around the mean therefore homogeneity of the sample can be assumed to be satisfactory and bacterial distribution in the sample resembles normal distribution
- Stability: colony counts of samples showed good stability for 5 to 7 days after dispatch when sample temperature is well controlled (6°C, TPC). This is assumed to be true for both samples as media and strains used were identical and a smaller number of analysis with sample A gave similar results (data not shown)

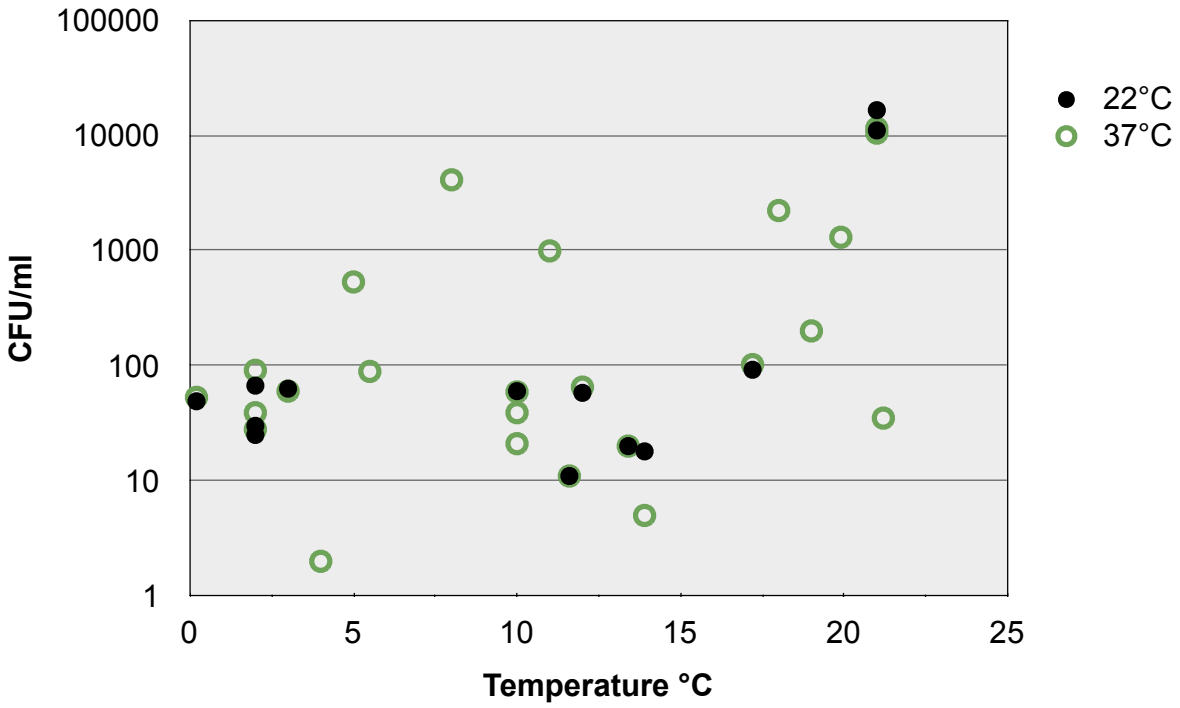
Logistics - Delivery times and temperatures



QC of results sample A



QC of results sample B



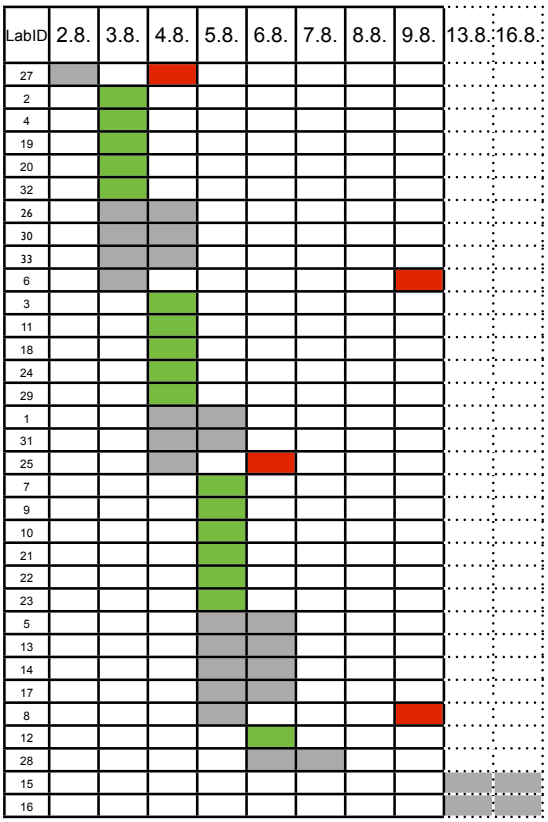
Logistics

- Delivery: 18 out of 33 samples reached their destination within the optimal 2 day period and 12 samples showed temperatures in the desired range at reception
- There is no correlation to be seen between sample temperature at reception and number of bacteria detected
- Packaging has to be optimized
- Delivery times were not met by DHL

Evaluation part 2: Participants

- General
- Sample (A and B)
  - Logistics
  - Results
  - Methods

Analysis - dates



Optimum: receipt of sample and analysis on the same day

Acceptable: analysis performed on the day after receipt

Not acceptable: delayed analysis (>24 h after receipt of sample)

Results

				Sample A		Sample B		
Lab ID	Delivery date	Date of analysis	Temperature at arrival (°C)	E. coli (CFU/100 ml)	Coliform bacteria (CFU/100 ml)	TPC 22°C (CFU/ml)	TPC 37°C (CFU/ml)	Comment
1	4.8.	5.8.	2,0		5	25	28	Delayed analysis
2	3.8.	3.8.	5,5	48	48		89	
3	4.8.	4.8.	12,0	33	33	58	65	
4	3.8.	3.8.	3,0	detected	30	63	60	E. coli result is no count; not a valid result for statistical analysis of the PT
5	5.8.	6.8.	21,0	95	95	16500	11.500	delayed analysis; TPC counts far too high (dilution/ miscalculation?)
6	3.8.	9.8.	5,0	detected	44		532	E. coli result is no count; not a valid result for statistical analysis of the PT; TPC count too high (dilution/miscalculation)
7	5.8.	5.8.	21,6				>200	TPC result is not a valid result for statistical analysis of the PT; correct according to ISO 8199; count too high
8	5.8.	9.8.	21,2	0	0		35	delayed analysis; E. coli and coliforms should have been detected
9	5.8.	5.8.	10,0	0	0		39	E. coli and coliforms should have been detected
10	5.8.	5.8.	10,0	0	0		21	E. coli and coliforms should have been detected
11	4.8.	4.8.	3,0	37	45			E. coli < Coliforms?
12	6.8.	6.8.	21,0		4600	11000	10430	All counts far too high (dilution/miscalculation?)
13	5.8.	6.8.	2,0	0	0	30	39	E. coli and coliforms should have been detected
14	5.8.	6.8.	1,0	34	34			
15	13.8.	16.8.		43		490	81	Very good results considering the delay in customs; discrepancy between TPC at different temperatures surprising
16	13.8.	16.8.	Chilled	820	820	16364	14455	All counts too high; delayed in customs
17	5.8.	6.8.	19,0	1.1	>23		200	E. coli / Coliforms? How do you detect 1/10th of an organism? Coliform result not a valid result for statistical analysis of the PT

Results

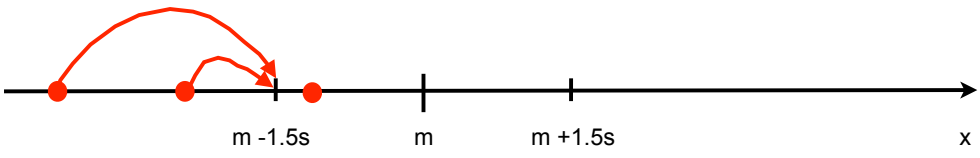
				Sample A		Sample B		
Lab ID	Delivery date	Date of analysis	Temperature at arrival (°C)	E. coli (CFU/100 ml)	Coliform bacteria (CFU/100 ml)	TPC 22°C (CFU/ml)	TPC 37°C (CFU/ml)	Comment
18	4.8.	4.8.	18,0	38	41		2219	TPC counts too high
19	3.8.	3.8.	0,2	115	115	49	53	
20	3.8.	3.8.	11,6	11	18	11	11	
21	5.8.	5.8.	13,9	>8	>8	18	5	„>A“ not a valid result for statistical analysis of the PT
22	5.8.	5.8.	13,4	200	200	20	20	
23	5.8.	5.8.	13,0	50	50			
24	4.8.	4.8.	17,2	60	0	92	102	E. coli > Coliforms?
25	4.8.	6.8.	20,7	1				Delayed analysis; very low number of E. coli
26	3.8.	4.8.	8,0	2.300	4300		4100	Delayed analysis; all results far too high (dilution/ miscalculation?)
27	2.8.	4.8.	11,0	130	130		990	Delayed analysis; TPC result too high (dilution/ miscalculation?)
28	6.8.	7.8.	19,9	16	16		1300	Delayed analysis; TPC result too high (dilution/miscalculation?)
29	4.8.	4.8.	-1,1	93	93		43	
30	3.8.	4.8.	10,0	90	95	60	59	Delayed analysis
31	4.8.	5.8.	2,0	0	1.4	67	91	Delayed analysis; how do you detect parts of a microorganism?
32	3.8.	3.8.	4,0	<100	<100		2	„<A“ not a valid result for statistical analysis of the PT; is the method fit for purpose? Detection limit of E. coli 100/100 ml?
33	3.8.	4.8.	<0		25	63	62	Delayed analysis

ISO 13528 - assigned value

- 1 possibility for use as an assigned value is to use a consensus value from participants
- advantage: easy to realize, cheap and particularly usefull with operationally defined measurement methods
- Disadvantage: biased if the results are biased
- There might be no consensus

ISO 13528 - AlgA

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



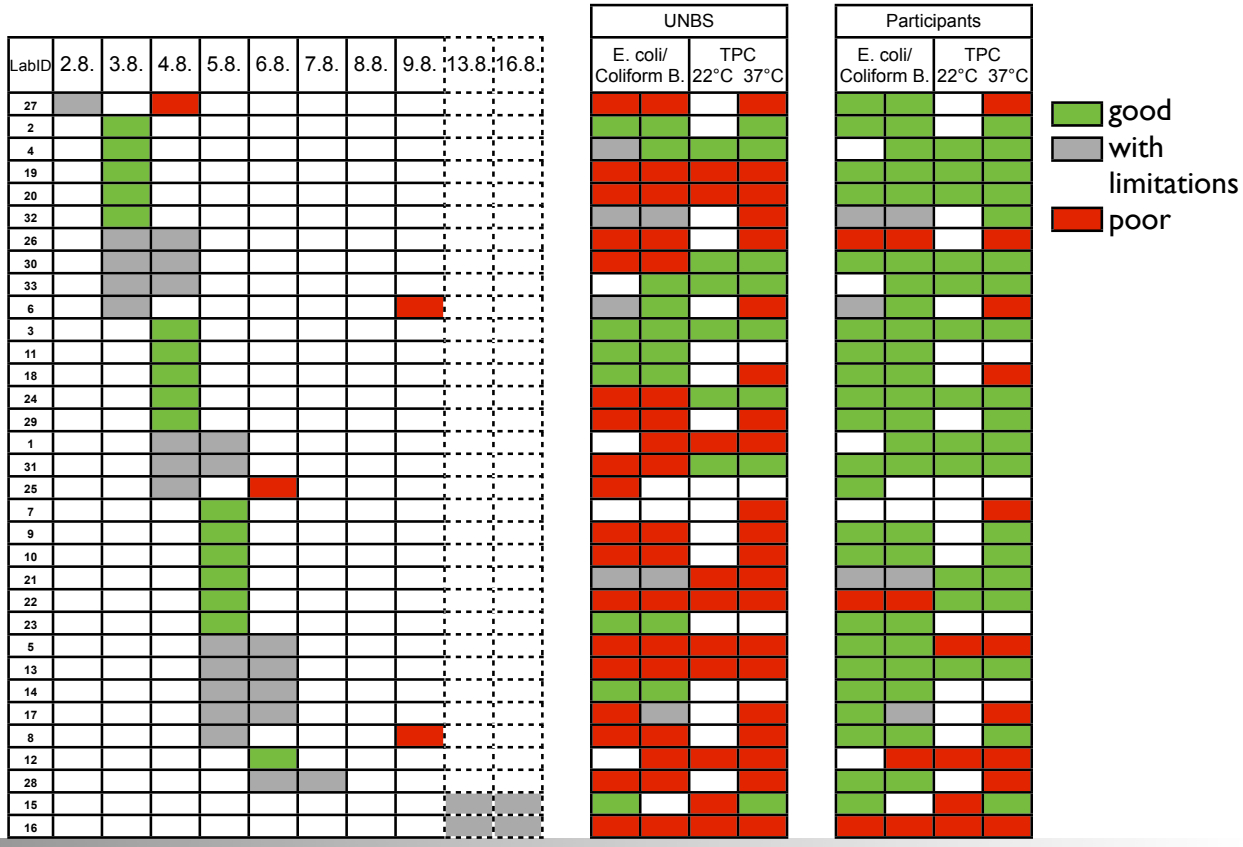
Reference values

Parameter	Data from	AlgA Mean	AlgA SD	Lower limit	Upper limit
E. coli	UNBS	36	7	22	50
	Participants*	45	53	0	151
Coliform B	UNBS	As for E. coli			
	Participants*	48	48	0	144
TPC 37°C	UNBS	79	12	55	103
	Participants*	58	43	0	144
TPC 22°C	UNBS	As for TPC 37°C			
	Participants*	50	32	0	114

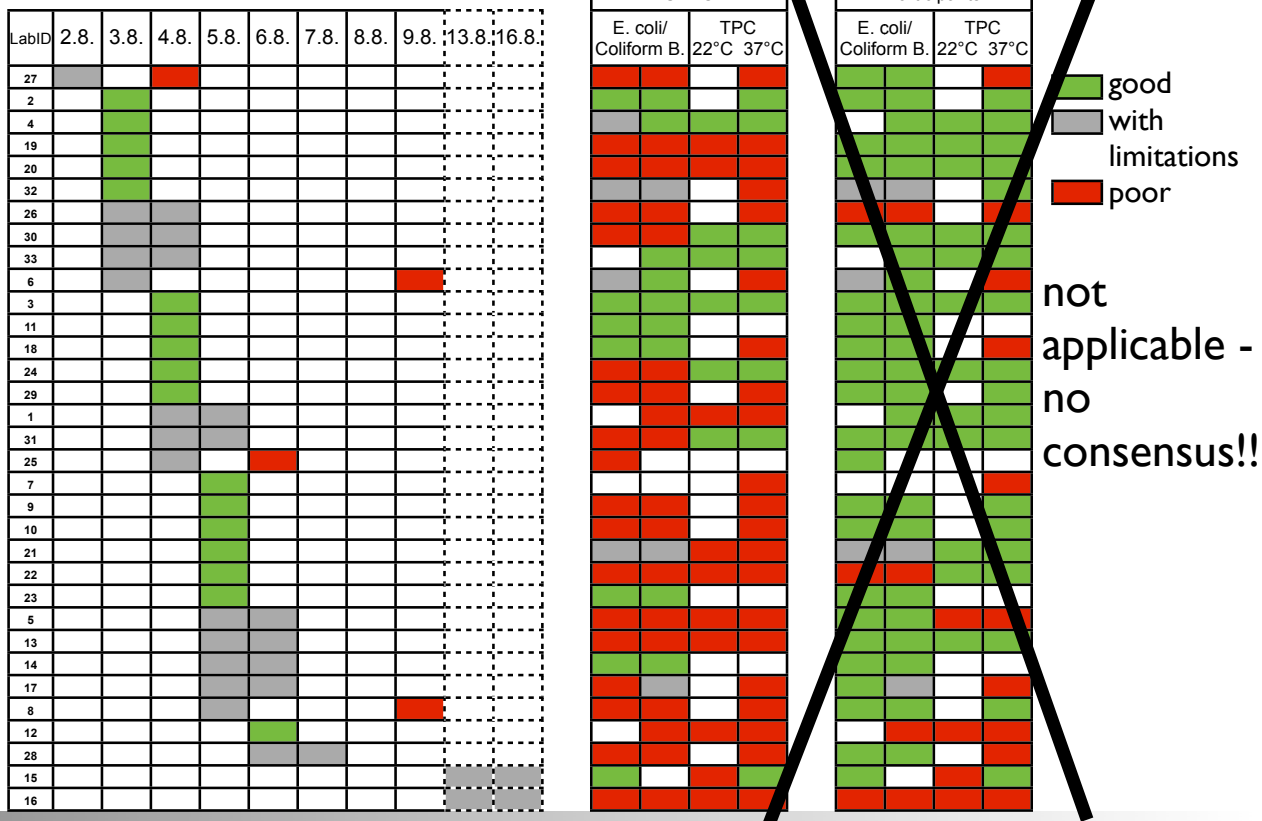
* obviously faulty results > 1000 were excluded from the calculation

AlgA is not a good method if there is no consensus value!
SD close to mean
large discrepancy between mean and median

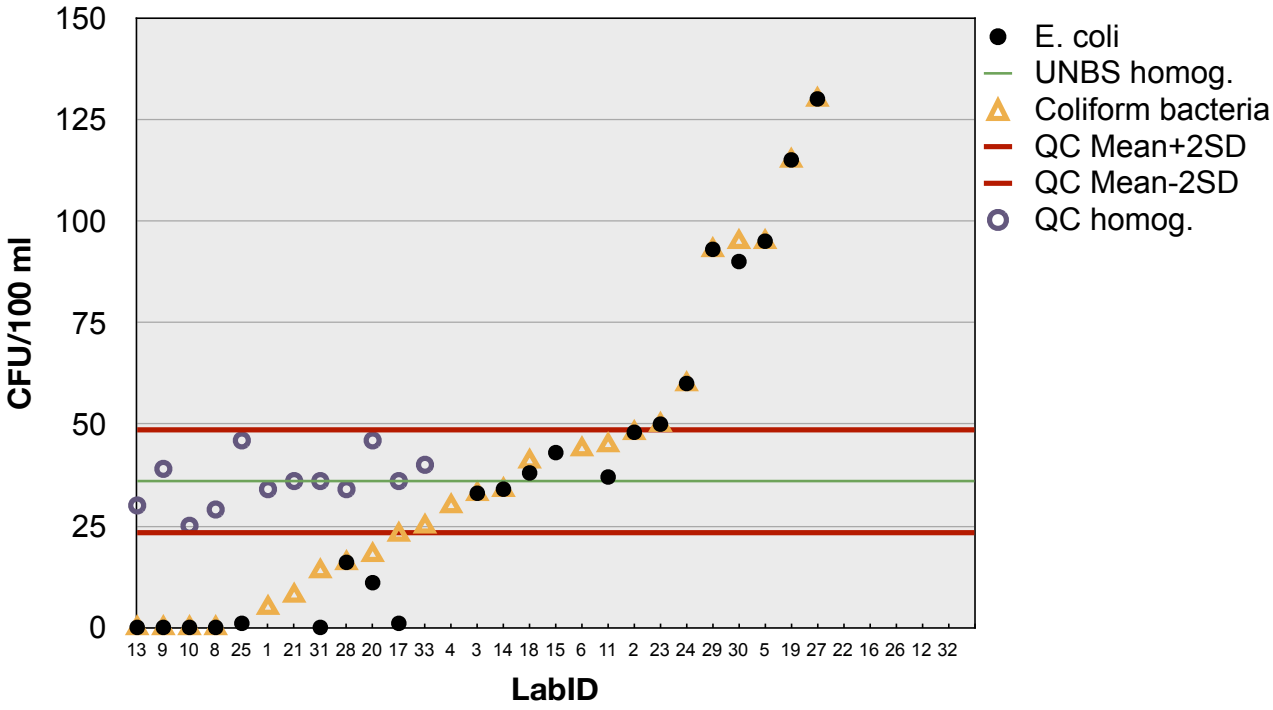
Performance (reference value derived from UNBS QC or participants data)



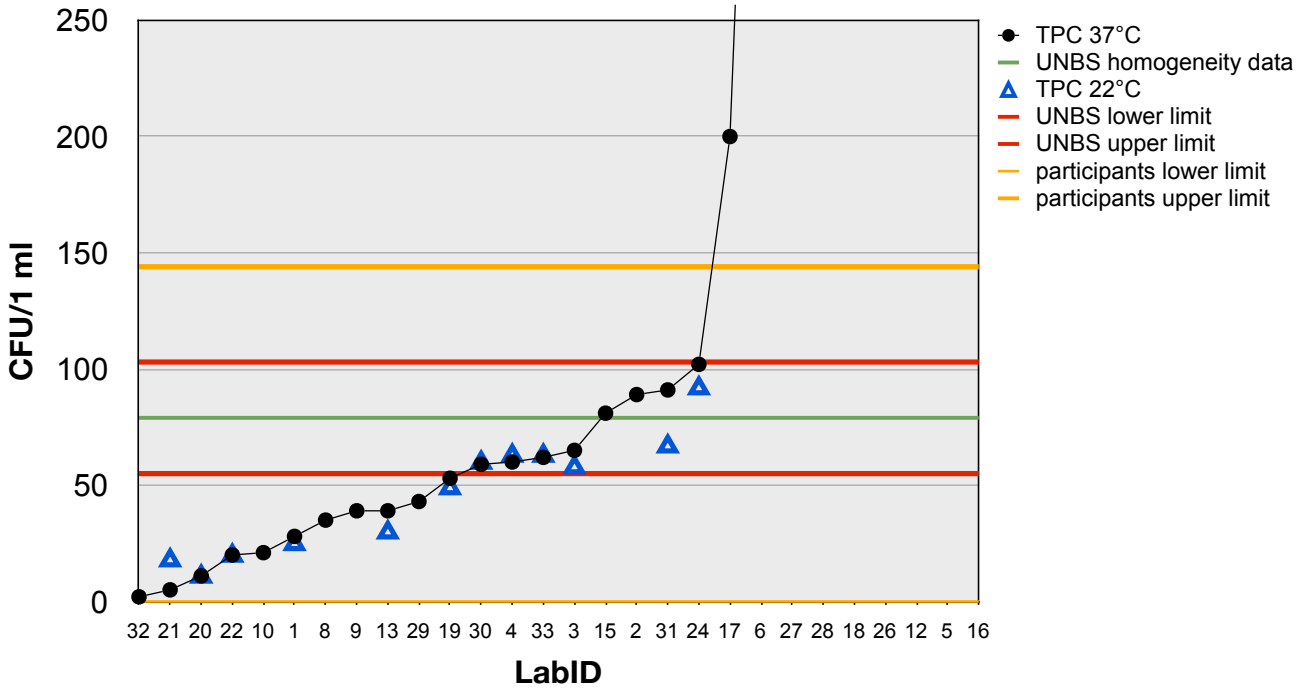
Performance (reference value derived from UNBS QC or participants data)



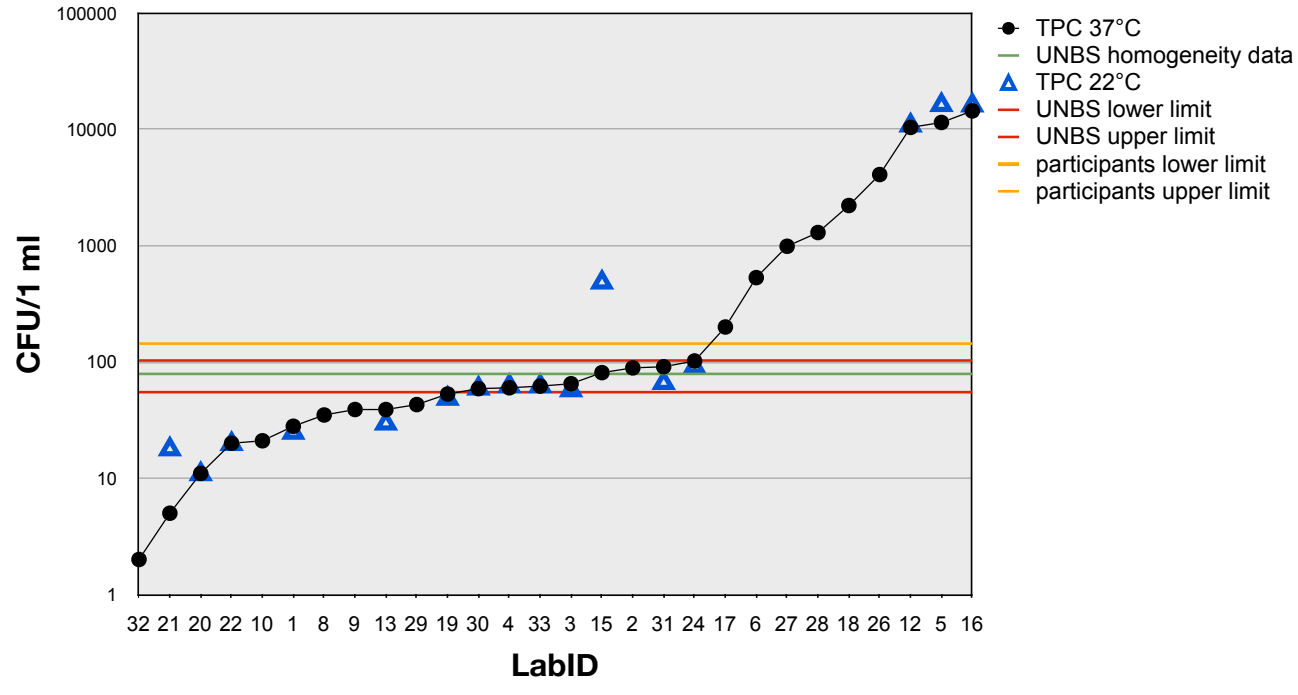
Results sample A



Results TPC



Results TPC



Results

				Sample A		Sample B		
Lab ID	Delivery date	Date of analysis	Temperature at arrival (°C)	E. coli (CFU/100 ml)	Coliform bacteria (CFU/100 ml)	TPC 22°C (CFU/ml)	TPC 37°C (CFU/ml)	Comment
6	3.8.	9.8.	5,0	detected	44		532	Sample A seems to be OK
27	2.8.	4.8.	11,0	130	130		990	Sample A slightly high
28	6.8.	7.8.	19,9	16	16		1300	Sample A rather low and sample B rather high
18	4.8.	4.8.	18,0	38	41		2219	Sample A seems to be OK
26	3.8.	4.8.	8,0	2.300	4300		4100	Delayed analysis; all results far too high (dilution/ miscalculation?)
12	6.8.	6.8.	21,0		4600	11000	10430	All counts far too high (dilution/miscalculation/growth?)
5	5.8.	6.8.	21,0	95	95	16500	11.500	Sample A slightly high; sample B much too high
16	13.8.	16.8.	Chilled	820	820	16364	14455	All counts too high; delayed in customs

Methods

Lab ID	Method used	Media used	Temperature and length of incubation
1	Membrane Filtration - BIOL/ CSES 4644	M - FC agar (Biolab)	24 hours at 44°C
2	IDEXX Colilert - 18 Quanti tray	Colilert 18 Reagent	18 hours at 35°C
3	RS - 13 : 2004; Ref. EAS: 217 -3; KS 220: 1972	VRB, BGB. (Kovacs for E.coli confirmation	48 hours at 30°C ; 44°C at 24 hours for E. Coli
4	TES /MIC/TM/14; TES/MIC/TM/13	VRB	24 hours at 44°C; 24 hours at 37°C
5	Membrane Filtration	VRBG	24 hours at 37°C
6	ISO 9308 -1	VRBL	24 hours at 37°C
7			
8	ISO 9308 -1: 2000	Tergitol - 7 Agar	24 hours at 37°C
9	Pour plate method	EMB & MacConkey	EMB - 24 hours at 44°C; MacConkey - 48 hours at 37°C
10	Pour plate method	Violet red bile agar	48 hours at 45°C and 37°C respectively
11	Colilert 18 - Quanti tray	Colilert 18 Reagent	18 hours at 37°C
12	As per manuals of Food Quality control, Microbiological Analysis; 14/4 Revision 1	MacConkey broth,Buffered peptone water, Brilliant green	24 - 48 hours
13	MPN (Colilert)		18 - 24 hours at 37°C
14	Colilert 18 - Quanti tray	Colilert 18 Reagent	20 hours at 37oC
15	MPN	Lauryl sulfate broth; Brilliant green bile lactose agar	Lauryl sulfate broth(48 hours at 37°C); Brilliant green bile lactose agar(24 hours at 37°C)
16	ISO 9308-1: 2000	Tergitol	24 hours at 37°C
17	ISO 9308 -2	Lauryl tryptose (lactose)broth; Brilliant green lactose(bile) agar; EC medium; Tryptose broth	Lauryl tryptose (lactose) broth(35°C - 48 h); Brilliant green lactose(bile) agar (35°C - 48 h); EC medium (44.5°C - 24 h); Tryptose broth (44.5°C - 24 h)

Methods

Lab ID	Method used	Media used	Temperature and length of incubation
18	Colilert	Quantitray	23 hours at 35°C
19	ISO 9308 -1	Lactose TTC agar	24 hours
20	KS05 -459 Part 3 : 1985 (MPN for coliforms & E.coli)	MacConkey broth - purple for coliforms at 37oC; Tryptone broth for E.coli at 44 Oc	Coliforms: 48 hours at 37°C; E.coli : 48 hours at 44°C
21	Colilert 18 (Enumeration of Total coliforms & E.coli)	Colilert 18 Reagent	19 h 30 min
22	Pour plate	TBX (Tryptone Bile X - Glucuronide agar) & VRBA - Violet Bile Agar (Red)	44°C & 37°C respectively
23	Colilert	Colilert 18 Reagent	20 hours at 35°C
24	Membrane Filter method (No.29)	On Coli blue 24 broth	24 hours at 35°C
25	Membrane filtration	M - FC Broth	24 hours at 44°C
26	ISO 7251 : 2005	Lauryl sulfate broth	48 hours at 37°C
27	9221	MacConkey; Brilliant green; Brilliant green; Tryptone water	MacConkey(48 h at 37°C); Brilliant green(48 h at 37°C); Brilliant green - Tryptone water (24 h at 44.5°C)
28	MFO -18	L.S.T, BGLB, LEMB	48 h at 37°C, 44°C
29	ISO 9308 - 2 : 1990	E.C. Broth; Brilliant green broth	44°C & 37°C respectively
30	ISO 9308 (2000)	M - Endo agar - les; M - FC - agar	24 hours
31	MPN	Lauryl Tryptose broth (For coliforms); Brilliant green Bile broth 2% (For E. coli)	37°C for 48 hours (for coliforms) and 44.5°C for 48 hours (E.coli)
32	US 2171 - 3: 2001; EAS 2171-3: 2001	Violet red bile agar (Hi media)	24 hours at 30°C
33	Membrane filtration	Endo	24 hours at 35°C

Assigned value - Chemistry

ISO/IEC Guide 43-1:1997

5 ways to determine an assigned v

- **known value** results determi specific test items
- **Certified referenc rials** as determined by definite metho
- **referenc es** – as determined by analysis, mea ent or comparison of the test item ide a reference material or standard, traceable a national or international standard

not available for microbiological samples

Assigned value - Chemistry

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

ISO 13528 - assigned value

- 1 possibility for use as an assigned value is to use a consensus value from participants
- advantage: easy to realize, cheap and particularly usefull with operationally defined measurement methods
- Disadvantage: biased if the results are biased
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ISO 13528 - AlgA

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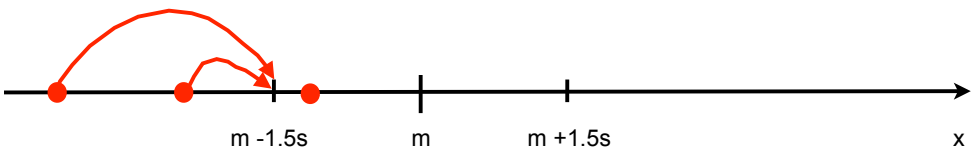


Working group discussion

- How should in future PTs the assigned value be determined?
- possibilities: Experts laboratories (how to define?) UNBS/ participants labs...
- please please make a list of pro/contra arguments
- come up with practical ideas

Results

- Participants not possible right now
- not so many expert labs UNBS Capacity/competency building at UNBS better develop / transportation improved
- accreditation of UNBS after 17043
- NLGA as expert lab for comparison to UNBS
- at least one expert lab should be in Africa to be confronted with the same problems as transport etc.
- maybe 2 or 3 expert labs (in Germany)
- raise awareness for importance of PT
- Randwater (SA PT provider) try to contact
- give two evaluations QC/expert lab data and participants
-

RECOMMENDATION OF TEST METHODS FOR DETECTION AND ENUMERATION OF COLIFORMS AND *ESCHERICHIA COLI* IN DRINKING WATER

Background

The 3rd Microbiology Proficiency Testing (PT) scheme evaluation workshop was held on the 1st to 4th November, 2010 in Windhoek, Namibia. During results analysis/discussions, it was observed that there were large variations in the results obtained by the participating laboratories. These variations were thought to be attributable to the various test methods used as much as to the performance of the laboratories in the PT scheme. This made it difficult to compare and perform statistical analysis. It is against this background that participants in the PT evaluation workshop came up with the following recommendation:

Recommendation

Participants recommended that internationally accepted test methods applicable to drinking water be used. The following test methods were recommended:

1. **ISO 9308 – 1: 2000 – Water Quality – Detection and enumeration of *E. coli* and coliform bacteria**
   This is a membrane filtration technique using Lactose TTC agar (Tergitol- 7). Typical Coliforms and *E. coli* produce galactosidase to ferment lactose resulting in acid production (yellow color under the membrane). The presumptive colonies are tested for oxidase and indole. Colonies positive for indole test are further tested for glucuronidase production to confirm them as *Escherichia coli*.

2. **Colilert – 18 ® - Enumeration of Total Coliforms and *E. coli***
   This is a commercially available Most-probable number (MPN) technique using enzyme-substrate liquid-broth medium that allows for simultaneous detection of total coliforms and *Escherichia coli* (*E. coli*). Two enzyme substrates; a chromogen that reacts with the enzyme found in coliform bacteria (galactosidase) and a fluorogen that reacts with an enzyme found in *E. coli* (glucuronidase).
   After 24 hours incubation at 37^{o}C, coliform bacteria turn the medium yellow and *E. coli*-positive reaction causes the medium to fluoresce under a long-wave ultraviolet light (366 nm).

Participants acknowledge that there may be challenges in adopting the recommended methods due to the unavailability of resources. However, the benefits of adopting these recommended methods are long term and outweigh the challenges that may be experienced when introducing the new methods.

Some of the Benefits of using the recommended methods

- Reducing technical barriers to trade;
- Assist new laboratories to correctly select appropriate methods for analysis of drinking water
- Facilitate the provision of technical collaboration and comparability of results among the SADC WATER LABORATORY ASSOCIATION members

For purpose of testing any kind of water for compliance with a limit of 0 *E. coli* in a given volume e.g. 100 ml, this volume should be used for analysis. An MPN technique using only a fraction of that volume should not be considered fit for purpose.

Feed back

Members are encouraged to direct any feedback and/or suggestions to the SADC secretariat at dmasuku@nmisa.org, Private bag X34, Lynnwood Ridge, Pretoria, South Africa.

----------§§ End §§----------

SADCMET Water PT Evaluation Workshop

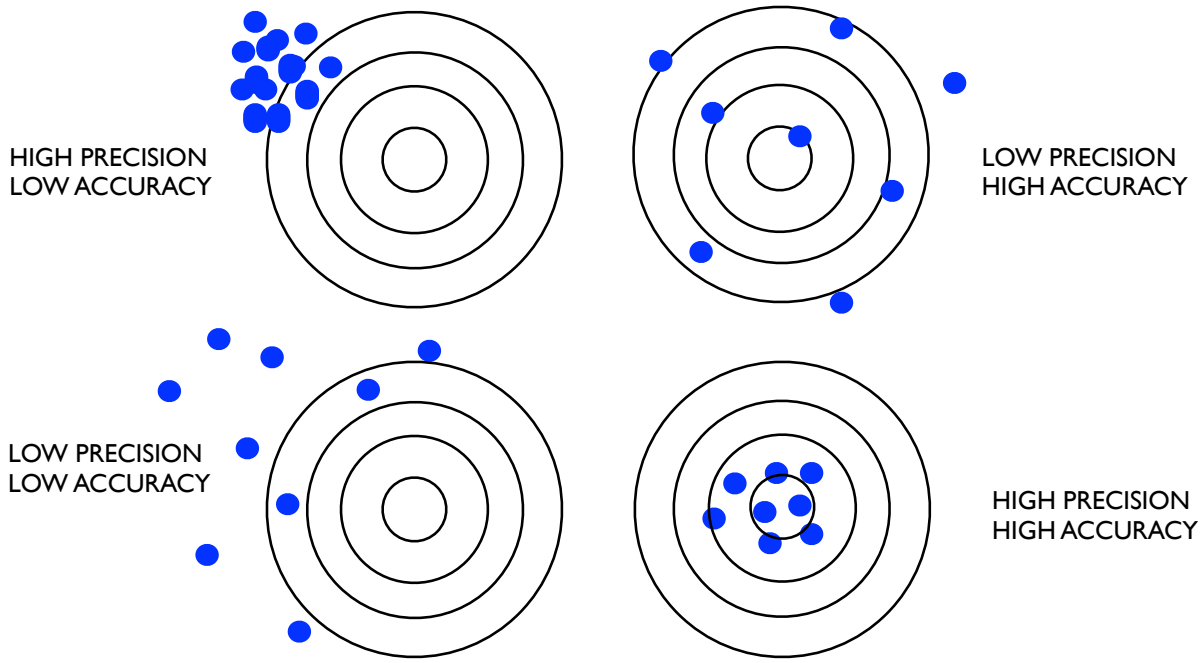
Validation of microbiological methods

(ISO/TR 13843:2000 Water quality - Guidance on validation of microbiological methods)

Method Validation

- Validation: process, giving proof that a method is fit for purpose
- Purpose: Detection of a specific microorganism (or a group of organisms) with sufficient precision and accuracy.

Precision and accuracy



VALIDATION

- Primary validation: Determination of specifications for using a new method and/or experimental verification that a method fulfills the theoretically derived quality criteria
- process of establishing operational limits and performance characteristics of a new, modified or otherwise insufficiently described method (performance specifications)
- Secondary validation (verification): experimental verification that an established method works in the hand of the user according to its characteristic values (establishes that the method performs to its specifications)

Performance specifications

- Morphological Identification of target organism
- Incubation conditions (time, temperature, humidity...) and media characteristics
- Media characteristics (pH, Stability...)
- working limits
- Measurement uncertainty

Unfortunately

- .... It is almost impossible to detect the true concentration of an microbiological sample
- Traceability is impossible
- No absolute recovery can be assessed
- ⇒ Relative recovery compared to a reference method becomes important
- Microbiological methods are not robust

Description of characteristics

		presumtive result		
		+	-	
confirmed results	+	a	b	a + b
	-	c	d	c + d
		a + c	b + d	n

Colonies should be picked randomly from all colonies (presumptive positives and negatives).
Due to the large influence of the person executing the experiment all these characteristics are not specifically constant for a certain method.

Definitions

		presumtive result		
		+	-	
confirmed results	+	a	b	a + b
	-	c	d	c + d
		a + c	b + d	n

a) true positives

b) false negatives

c) false positives

d) true negatives

Method characteristics

		presumtive result		
		+	-	
confirmed results	+	a	b	a + b
	-	c	d	c + d
		a + c	b + d	n

sensitivity = a/(a+b)

specificity = d/(c+d)

false positive rate = c/(a+c)

false negative rate = d/(b+d)

efficacy E = (a+d)/n

Performance characteristics

- Sensitivity > 90 % of presumptive positives should be confirmed (exceptions named)
- Selectivity = presumptive colonies/total number of colonies F = lg(t/n) F > -1 less than 2 is not a

Practical approach

- Determine relevant scope that the method is supposed to fulfill
- pure culture experiments to come up with a basic desription of colonies of your target organisms (use more than 1 strain!)
- use natural samples for comparison
- basic reliability should be tested by recounting the same plates by different persons

Practical approach

- calculation of numerical values for
  - sensititivity
  - selectivity
  - false positives
  - false negatives
  - efficency
- incubation time, temperatures...

Uncertainty

- Uncertainty of counting: repeat counting of the same plate by the same person (RSD)
- Uncertainty of counting (different persons)
- Uncertainty (dependence on time) repeat counting at beginning and end of designated incubation time (RSD)
- important: calculate and judge these data

- this allows for first idea about were the problems of the method might be in routine use

Robustness of the method

- Influence of incubation temperature, humidity, gasatmosphere ... should be experimentally calculated if necessary
- factors assumed to be of little or no influence do not need to be checked in experiments

Proportionality/Linearity

- upper limit of the working range of the method can be determined by a linearity test: dilute a sample 1:2:3:4:5:6:7 or 1:2:4:8:16:32:64 count three replicates each and analyse the ratio no of colonies/relative volume

Linearity

Dilution	replicate counts			sum Si	relative volume Ri	ratio Si/Ri
2^{-1}	121	204	162	487	32	15,22
2^{-2}	109	128	148	385	16	24,06
2^{-3}	111	114	97	322	8	40,25
2^{-4}	56	60	68	184	4	46,00
2^{-5}	36	29	24	89	2	44,5
2^{-6}	11	13	17	41	1	41,00
				Total 1508	63	

Linearity can be judged by Log-likelyhood quotientindex G^2

Linearity not given for counts >120/plate

Example for validation experiment

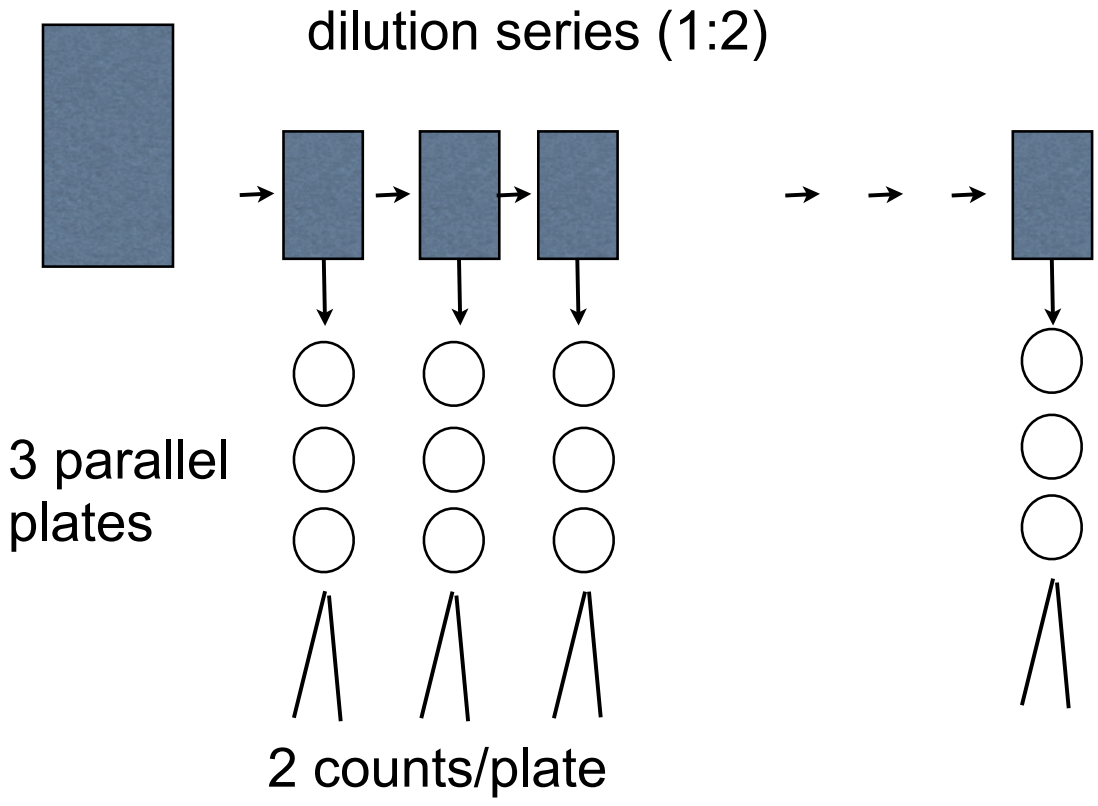
for colony count method and plating

suspension - dilution series (7 dilutions)

every dilution plated 3 times (replicates) - counting repeated by 3 different persons

repeat this for differen G^2 t infuence factors

Membrane filtration: use different volumes e.g. 1,2,3,4,5,6 ml per filtration



SADCMET Water PT Evaluation Workshop

Measurement uncertainty

Different Approaches (Chemistry)

- **Top-Down Approach** (GUM/ EURACHEM/CITAC)
- **Bottom-UP Approach** (**V**alid **A**nalyitkal **M**easurment/NORDTEST) Estimation of Measurment uncertainty using accuracy and precision (PT, CRM, Controlcharts) Microbiologie: Repeatability experiments with reference material from PTs?
- simplified method by EUROLAB estimate of MU using SD of PT data for

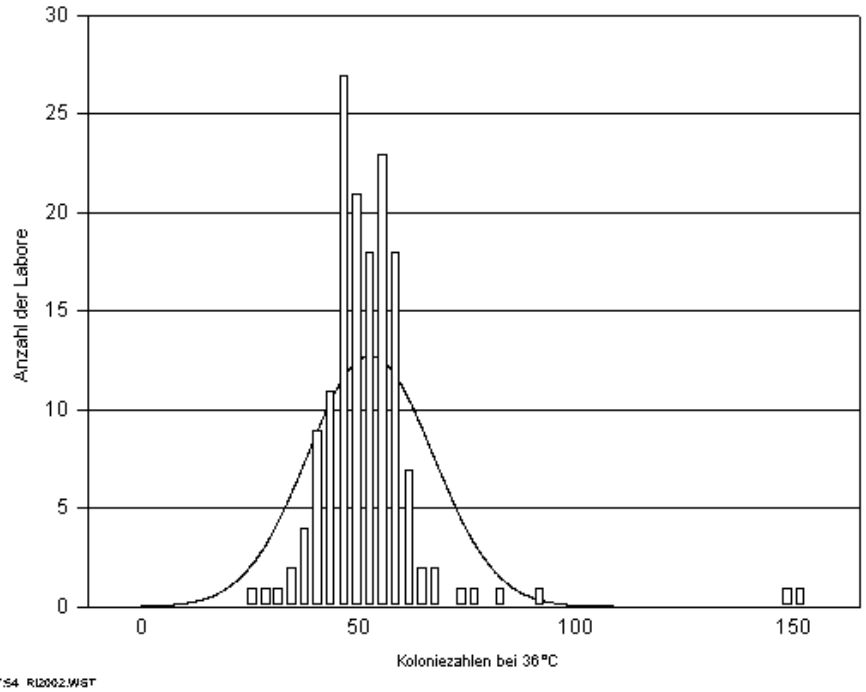
ISO CD 29201

The variability of test results and the uncertainty of measurement of microbiological enumeration methods

2 ways:

step-by-step (GUM)

global approach



GUM Legionella

Sampling conditions temperature, transport times, light, influence of other microorganisms

pipetting, subsampling (2x 0.5 ml out of 100 ml) Homogenisation Adsorption to pipette tips or bottles

filtration: volume measurement acid treatment HCl/KCl buffer pH 2.2 +- 0.2

results: counting identifiying of target organisms confirmation

$$u_c(y) = \sqrt{u_{x1}^2 + u_{x2}^2 + u_{x3}^2 + ... + u_{xn}^2}$$

Media: humidity storage conditions thickness of layer ingredients interaction with membranes

spreading of sample (adsorption to Spreading device) Influence of staff

incubation conditions: temperature change in media (7-10 days) bacterial interaction Synergism / Antagonism

Reference material (certified?)

VAM

- MU calculated using precision and accuracy data e.g. from PT schemes
- Accuracy: reference material consensus mean of PT samples
- Precision: multiple analysis of the same material e.g. TPC method within the own laboratory - examples given for NLGA (QC analysis from PT schemes)

VAM

combined uncertainty:

$$u_c(y) = \sqrt{s_{rel}^2 + u(\overline{R}_m)^2}$$

s= RSD = s_{obs}/c_{obs}
measurments with RM

relative uncertainty of the mean Recovery rate of the reference material:

$$u(\overline{R}_m) = \overline{R}_m \bullet \sqrt{\left(\frac{s^2}{n \bullet \overline{c}_{obs}^2}\right) + \left(\frac{u(c_{CRM})}{c_{CRM}}\right)^2}$$

Rm = relative Recovery rate C_{obs}/C_{CRM}
n = number of results
C_{obs} = mean concentration of repeat analysis

VAM

extended MU: k=2

$$u_c(y) \bullet 2$$

Gluschke, Wellmitz & Lepom:

A case study in the practical estimation of measurement uncertainty.

Accred.Qual Assur (2005), 10:107-111

VAM Example

data derived from PT samples (provided with the report:

Labordaten aus dem NLGA AS Aurich

KBE 20°C (20-iger Test-RV Labor Aurich RV 3 2005 Gr A)												N	C_{obs}	s_{obs}	C_{CRM}	$u(C_{CRM})$	RSD	R_m	$u(R_m)$	t	$u_c(y)$	U(y)	**U(y)**
6	6	4	10	5	9	8	11	6	7	10	10	20	7,2	2,931	8,0	2,200	0,407	0,90	0,261	0,384	0,483	0,967	**+-96,7%**
7	14	2	4	4	9	5	7																

Eingabe nur in gelb unterlegte Felder! Berechnungen basieren auf unserer Leitlinie!

- n number of replicates in the lab, without outliers
- C_{obs} mean of replicate analyses of the Certified Reference Material (CRM)
- s_{obs} standard deviation of the results from the replicate analyses of the CRM
- C_{CRM} certified concentration of the analyte in the CRM; see the certificate provided by the producer of the CRM
- $u(C_{CF}$ standard uncertainty of the certified concentration for the CRM; see certificate provided by the producer of the CRM
- RSD relative standard deviation
- R_m mean recovery
- $u(R_m)$ relative uncertainty of the recovery
- t test statistic; see our case study
- $u_c(y)$ combined uncertainty
- U(y) expanded uncertainty, k=2; see our case study

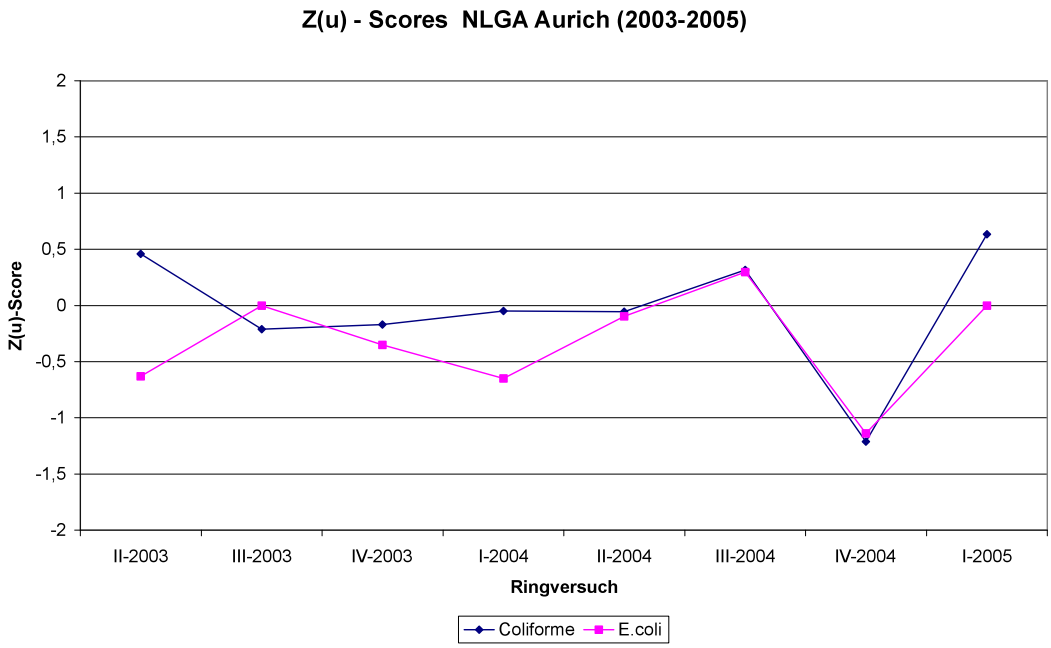
EUROLAB

simplified method by EUROLAB using RSD from PT schemes e.g. 3,4 %

applicable only under the following conditions:

- Laboratory has to have participated in the respective PT
- intralaboratory RSD must not be larger than RSD of PT
- laboratory must have established a functioning QC system
- controlcharts have to be used to demonstrate that quality

Controlchart example NLGA



Examples NLGA /German PTS

RV	Parameter	c (PT)	S (PT)	C^{QC}-20	S^{RV}-20	MU (VAM)	MU (EUROLAB)
2-04-B	TPC 20°C	84	10,1	102	8,4	34 %	24 %
1-05-A	TPC 20°C	12	2,5	13	3,2	68 %	42 %
3-05-A	TPC 20°C	8	2,2	7	2,9	97 %	55 %
1-04-B	TPC 36°C	79	8,5	94	11,5	35 %	22 %
4-05-A	Leg. 1 ml	14	10,3	16	3,2	175 %	147 %
4-05-B	Leg. 1 ml	22	13,8	22	4,7	137 %	126 %
4-05-A	E. coli DIN	6	3,2	9	3,3	185 %	107 %
4-05-A	P. aeruginosa	19	5,7	18	4,4	76 %	60 %
4-05-A	IEK	30	7	28	8,2	74 %	47 %
3-05-A	C. perfringens	30	13,1	33	7,4	107 %	87 %

Evaluation questionnaire - Microbiology workshop Windhoek

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
Report of PT provider	☐	☐	☐	☐	☐
The evaluation of the PT	☐	☐	☐	☐	☐
Discussion on methods	☐	☐	☐	☐	☐
Method validation	☐	☐	☐	☐	☐
Measurement uncertainty	☐	☐	☐	☐	☐
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?

Participants list

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