A GUIDE ON INTERNAL QUALITY CONTROL AND QUALITY ASSURANCE FOR TANZANIA- NATIONAL FISH QUALITY CONTROL LABORATORY

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ABSTRACT

Results from laboratories are important to facilitate import and export of goods including food. It is therefore of prime importance that the laboratory is capable of performing its tasks and accreditation is one way of determining that. The ISO 17025 standard describes the "general requirements for the competence of calibration and testing laboratories". Internal quality control and quality assurance routines play a major role for the quality of results. The National Fish Quality Control Laboratory (NFQCL) in the Lake Victoria Zone is working towards achieving an accreditation of its quality system in accordance with the ISO 17025 standard. The purpose of this study is to identify and obtain experience in IQC and QA routines. For that purpose a list of internal QC/QA routines were established and quality control charts were applied to follow the trends of microbiological results. To obtain analytical measurements and results that are fit for this purpose, samples must be collected and handled correctly. The measurement methods must be validated and effectively controlled, suitable calibration and control standards must be used and all personnel involved in the analytical activities must be qualified and competently trained. Microbiological examinations on *total plate counts*, *coliform* organisms, *Enterobacteriaceae*, *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes* were performed. Samples were spiked with pure strains of *Staphylococcus aureus*, *Salmonella* and *Listeria monocytogenes*. Spiking was done to increase the possibility to follow the test methods from the start to the end to fulfil the validation of methods and procedures. The QC/QA routines listed and the experience gained will benefit the work of building a quality system at the NFQLC in Tanzania.

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ABBREVIATIONS

A2LA	American Association for Laboratory Accreditation
AE/AEL	European Cooperation for Accreditation of Laboratories
AOAC	Association of Official Analytical Chemists
BGA	Brilliant green agar
BGBLB	Brilliant green bile lactose broth
BHI	Brain heart infusion medium
BPA	Baird Parker agar
BPL	Baird Parker liquid
BSA	Bismith sulfite agar
CA	Competent authority
CCM	Colony count method
CFU	Colony forming unity
CRC	Certified reference culture
EC	Escherichia coli medium
EEB	Enterobacteriaceae enrichment broth
EU	European Union
FBM	Fraser broth medium
HACCP	Hazard analysis critical control point
HEA	Hektoen enteric agar
IEC	International Electronic Commission
IQC	Internal quality control
ISO	International Organization for Standardization
IUPAC	International Union of Pure Chemistry
LST	Laurly sulfate tryptose broth
MOX	Oxoid agar modified
MPN	Most probable number
NA	Nutrient agar
NFQCL	National Fish Quality Control Laboratory
NMKL	Nordic Committee for Food Analysis
QA	Quality assurance
QC	Quality control
RM	Reference materials
RVB	Rapport vasiliadis broth
SCB	Selenite cystine broth
SWEDAC	Swedish Board for Accreditation and Conformity Assessment
TCBS	Thiosulfate bile salt sucrose agar
TSA-YE	Trypticase soy broth yeast extract
UVM	University of Vermont broth medium
VRBA	Violet red bile agar
XLD	Xylose lysine deoxycholate agar

1   INTRODUCTION

1.1   Background information

General requirements for the competence of testing and calibration laboratories accreditation are laid down in ISO/IEC 17025 (2005). One way of accepting test results is that the laboratories document the quality system underlying certain recognised standards. International markets for fish export require both legislation and infrastructure to be of the same standards as those applicable in their countries i.e. the European Union (EU) market. The export of various foods including fish products requires certified test results from recognised institutions i.e. accredited laboratories, especially regarding parameters of food safety concern including microorganisms and chemical contaminants. Fish (food) producers should also have an access to accredited laboratories.

The meaning of the terms 'Quality Control' (QC) and 'Quality Assurance' (QA) often vary according to the context (CITAC 2002). In practical terms, QA relates to the overall measures taken by the laboratory to regulate quality. Laboratory quality control is an essential aspect of ensuring that data released are fit for the purpose. For instance where performance falls outside an established range, data produced can be questioned and investigated for the cause of deviation and action taken to correct the deviation. The objective of laboratory quality assurance is to verify the accuracy and precision of information obtained from analytical results and to ensure that data are suitable for decision-making. The accuracy required depends on how the data are to be used.

The Tanzania Fisheries Division is the designated competent authority (CA) by the EU for all matters pertaining to fish and fishery products. This is in accordance with the Fisheries Act No. 22 of 2003 and Fisheries Regulations of 2005. The Fisheries Division as a competent authority is responsible for monitoring, surveillance, quality control and certifying fish and fishery products to meet national and international quality standards. In 1997 the Tanzania Fisheries Division established a National Fish Quality Control Laboratory (NFQCL) at the Lake Victoria Zone due to pressure from the EU in respect to export of Nile perch from Lake Victoria. The role of the laboratory is to verify the effectiveness and efficiency of quality assurance management systems in fish processing factories around the Lake Zone to ensure that they meet the requirements of international export markets. The NFQCL is well equipped for microbiological testing both with regard to personnel and facilities. Currently the NFQCL is in the final stages of finishing a new modern laboratory building, which will be equipped for both microbiological and chemical testing. This laboratory will be the biggest in Eastern and Central Africa in the fish quality assurance management sector.

The NFQCL is offering several services on microbiological analysis, namely total plate count, total coliforms, *Escherichia coli, Enterobacteriaceae, Salmonella, Vibrio cholerae* and *Listeria monocytogenes*. The NFQCL has started to implement a quality system in line with the ISO 17025 towards the process of receiving an accreditation. The laboratory lacks technological know how on how to fully implement this requirement. In particular, adequate documentation and method validation; internal audit and management reviews; use of certified reference culture and participating in proficiency testing. Currently the laboratory management is training personnel through seminars, attachments to accredited laboratories, short courses and even advanced learning to degree levels. This type of

training is expected to improve QC and QA routines. The implementation of efficiency and effective quality control routines are important for accreditation.

The ISO 17025 is divided into two main sections: management and technical requirements. The management requirements are of concern to:-
- the organisation,
- the management system,
- document control,
- review of requests, tenders and contracts,
- subcontracting tests and calibrations,
- purchasing services and supplies,
- service to the clients,
- complaints,
- control of non-conforming testing,
- improvement,
- corrective actions,
- preventive actions,
- control of records,
- internal audits, and
- management reviews.

The technical requirements include:-
- personnel,
- accommodation and environmental conditions,
- test and calibration methods and method validation,
- equipment,
- measurement traceability,
- sampling,
- handling of test and calibration items,
- assuring the quality of tests and calibration results, and
- reporting the results.

Technical requirements directly influence the quality of tests in the laboratory, which need efficient and effective international QC and QA routines throughout the analytical processes.

1.2 Rationale

The demand for high value commodities, safe to consumers is ever increasing in the international trade in food commodities including fish and fish products. This had gone through evolutions and applications of various international standards in particular the ISO 9000 family series for the total quality management of production. The Hazard Analysis for Critical Control Points (HACCP) and traceability to ensure food safety. The ISO 22000 for food safety management systems – includes requirements for any organisation in the food chain. Often the CA inspection bodies and laboratory services have to check the quality and safety of products prior to being certified for target use, export or import. The food export and import inspection and certification are required to have an accredited body, such as a laboratory, in order to demonstrate or prove that adequate quality controls are in place to ensure the reliability of the test results.

In Tanzania quality control (QC) and quality assurance (QA) systems in laboratories are not well established. However, the export of food products from Tanzania, as from any other country, to world markets are subject to conditions that they must be checked and inspected by accredited institutions. In order to certify a product it must be checked and found to meet the appropriate standards. Currently, the microbiological test results from fish and fish products are accepted by EU markets. In addition, fish processing factories are required to implement quality assurance management systems according to HACCP principles. Most of the processing plants have adopted such systems and they are regularly inspected by fisheries inspectors. In order to fulfil the requirements of international markets, other samples are sent to an accredited laboratory abroad for chemical tests and other parameters, which is expensive and getting the results takes time.

The Fisheries Division has been taking into consideration the issue of accrediting its laboratory to comply with international standards to avoid the excessive costs of analysing the samples outside the country. However, there is still a great need to harmonise the quality system in line with the ISO 17025. Efforts to develop and document the guidelines have been initiated. Lack of an efficient and effective quality system is the result of inadequate resources in terms of awareness, knowledge, material and technology. The development of the quality control guide during this study will facilitate the process of accreditation for the Tanzania National Fish Quality Control Laboratory. In addition, the implementation of the guide will enhance competency, confidence in the products and reliability of services offered to the customers as well as compliance to national, regional and international standards.

1.3 Scope, objective and goals

The scope of this project was "to set up and validate an internal quality control and quality assurance programme for test results in the food microbiological laboratory in line with ISO 17025". The general objective was to set guidelines for internal quality control and quality assurance for the Tanzania NFQCL. The specific goals were to familiarise, list and set up internal quality control and quality assurance routines, and follow analytical trends of quality assurance. The study was carried out at ProMat (food) Laboratory, Akureyri, Iceland. The ProMat Laboratory is accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC) for food microbiological testing.

2 MATERIAL AND METHODS

2.1 Setting internal QC and QA guidelines

General procedures that were used in setting the IQC and QA guidelines included the study and analysis of technical requirements for testing laboratories as detailed in ISO 17025:2005 second edition. Together with the ISO 17025 various guidelines proposed, prepared and used by accreditation bodies and food testing laboratories by the European Cooperation for Accreditation of Laboratories (AEL), the American Association for Laboratory Accreditation (A2LA), the Nordic Committee of Food Analysis (NMKL) and other citations. The knowledge obtained from the studies was harmonised with the quality control routines and practically applied by participating in daily activities and analyses performed in the food microbiology laboratory.

2.2 Quality assurance of results

2.2.1 Sample preparation

A total of nine samples, where seven were salted fish samples, were prepared and stored frozen. Two of them were control samples. Three types of sample materials were analysed during familiarisation of the laboratory quality system in food microbiological analyses. The first sample was a certified routine quality control sample analysed on a monthly basis to verify the internal quality control and assurance in the laboratory. The *Salmonella* and *Listeria monocytogenes* spiked samples. The last sample was the routine laboratory sample spiked with *Staphylococcus aureus*. Thirty-nine (39) replicate samples were analysed from 19 December until 2 February 2006.

2.2.2 Microbiological analysis procedures

The microbiological analysis procedures include colony count, surface spread, Most Probable Number (MPN) techniques and the specific microbiological test procedures for *Total plate count, Coliforms, Enterobacteriaceae*, *Staphlococcus aureus*, *Salmonnela* and *Listeria monocytogernes* (Appendix 1) (APHA1992) and in-house test procedures. The media used in the analyses were manufactured by DIFCO.

2.2.2.1 Colony Count Method (CCM)

The procedure is based on the assumption that each microbial cell will form a visible and separate colony by mixing with an agar or other solid medium and allowed to grow. The colony counts are reported as an estimate. Some microorganisms may not be capable of growing at the specified conditions. Microorganisms grow best at certain conditions i.e. at an optimum temperature and time (Swanson *et al*. 1992). According to Williams (1984) and Richardson (1985), CCM are based on pour plate and surface or spread techniques. The procedures are described as "standard plate count", "aerobic plate count", "total plate count", "viable plate count", "mesophilic count", etc. The process is often described based on the type and requirement of the sample being tested. Here, the total plate count method was used.

2.2.2.2 Most Probable Number (MPN)

An MPN was estimated from responses and results reported as positive or negative in one or more decimal dilution of the sample. In general, only three and five tubes MPN tables are published. The three series MPN tables were used. Peeler *et al*. (1992) stated that MPN is the best method on samples with microbial concentrations less than 10 organisms or Colony Forming Units (cfu/g). It is particularly important in the standard coliform procedure associated with water and food testing.

2.2.2.3 Enumeration of microorganisms

The sample homogenates was inoculated into plates followed by pouring molten (45±1°C) plate count agar. Mixed thoroughly and allowed to solidify and incubated at 30°C for 48 hours.

2.2.2.4   Examination of coliform organisms

Presumptive test for coliform by MPN method:

Four replicate tubes of lauryl sulphate tryptose (LST) broth were inoculated by 10 mL (double strength) then with 1 mL of sample homogenate decimal dilutions (1:1, 1:10, 1:100, 1:1000 or higher dilutions). Tubes were incubated for 48 hours at $35 \pm 0.5°C$. Tubes were read for gas production after 48 hours. The LST tubes that showed positive gas formation within 48 hours were recorded. Results were tabulated from MPN tables and reported as presumptive coliform bacteria (MPN /g or mL).

Confirmed test for coliforms:

Positive LST were subcultured into brilliant green bile (BGB) broth by means of a 3 mm loop and incubated at $35° \pm 0.5°C$ for 48 hours. BGB tubes that showed positive gas formation within 48 hours were recorded. Results referred to MPN tables and were reported as confirmed coliform bacteria (MPN/g or mL).

Escherichia coli:

Positive LST tubes were subcultured into EC broth by means of a 3 mm loop and incubated in a circulating water bath at $45.5 \pm 0.2°C$ for 48 hours. Positive EC broth tubes were referred to MPN tables.

2.2.2.5   Presumptive test for *Enterobacteriaceae* by pour plate and overlay

A tenfold serial dilution was prepared by using butterfields diluents' (1:10; 1:100; 1:1000 or higher dilutions). One (1 mL) of decimal dilutions was inoculated into replicate labelled plates. About 10 mL of molten ($45\pm1°C$) Violet Red Bile Glucose Agar (VRBGA) was poured and allowed to solidify. Overlaid with about 10 mL with VRBGA and allowed to solidify and incubated at $35°C$ for 18 to 24 hours.

2.2.2.6   Enumeration of Staphylococcus aureus

From the prepared sample homogenate 1 mL was inoculated in two plates each approximately 0.5 mL and was spread over the surface of solidified 110 medium by using sterile, bent glass spread rods. Plates were kept upright until the inoculum was absorbed by the medium and incubated for 72 hours at $35°C$. Plates containing 20 to 200 colonies were selected and counted. Several colonies of different types counted were tested for coagulase positive or clump factor. Positive coagulase or clump cultures were considered confirmed *Staphylococcus aureus*. Total numbers of counted colonies from duplicate plates were reported as *Staphylococcus aureus* (cfu/g or mL).

2.2.2.7   Detection of *Salmonella*

A sample was mixed and homogenised together with the Lactose broth pre-enrichment medium and incubated at $35°C$ for 24 hours. 0.1 mL was transferred into a secondary enrichment RV medium and 1 mL into TT broth and incubated for 24 hours at $35°C$. Streaked a loop full onto plates of BS, HE and XLD agars and incubated for 24 hours at $35°C$. All positive plates were streaked into TSI followed by LIA slants and incubated over

night at 35°C. Positive TSI and LIA tubes streaked onto NA slants to purify colonies for serological confirmation.

2.2.2.8   Detection of Listeria monocytogenes

A sample was mixed and homogenised together with the UVM pre-enrichment broth and incubated at 30°C for 24 hours. 0.1 mL was transferred into secondary enrichment fraser broth and incubated for 24 to 48 hours at 35°C. Black positive tubes were streaked onto MOX plates by using a sterile loop and incubated at 35°C for 24 to 48 hours. Five colonies from black MOX positive plates were streaked onto TSA-YE in separate sections and incubated for 24 hours at 35°C. Colonies from TSA-YE were confirmed by catalase test (H_2O_2) and gram colouring. Five colonies were transferred into 2 mL BHI tubes and incubated over night at 22°C room temperature and tested for motility.

2.2.2.9   Preparing control charts

The results were used to prepare control charts. The following steps were followed to make control charts:
* Established $\log_{10}$ for microorganism counts and mean value (mv).
* Standard deviation (s) calculated
* Control limits, the warning (mv±2s) and control (action) limits (mv±3s) were calculated
* mv $=?x/n$
* s $=v?(x-mv)^2/n-1$
* mv   = mean of the analyses data
* x   =data for each specific analysis
* n   =number of analysis/ replicates
* s   =standard deviation of the analyses

3   TECHNICAL REQUIREMENTS OF ISO 17025

This review focuses on ways applicable to the Tanzania National Fish Quality Control laboratory in implementing its internal quality assurance in line with ISO 17025. The specific review concerns the technical requirements of ISO 17025 and some aspects of the management requirements relating to the implementation of analytical quality assurance on testing work.

3.1   Personnel

(ISO 17025: clause 5.2)

The overall quality and performance of a laboratory is influenced by its personnel. Laboratory personnel's educational qualifications, practical experience and competency have to be demonstrated when required. The personnel have to understand the importance of quality assurance and their role in the process. They have to receive specific basic training for the competent performance of tests, methods/techniques and operation of equipment. Orientation and training for new analysts/personnel on the concept of quality control through an on-the-job training programme and a performance appraisal for all personnel is an essential element of the quality assurance system. The quality of test result

interpretation is directly connected to the experience of the laboratory personnel (analysts). The interpretation of test results for identification and verification of microorganisms is important. In some cases, it may be appropriate to relate competence to a particular technique or equipment rather than to methods. For this reason personnel shall be evaluated for performance on a regular basis and retraining provided when necessary.

Microbiological testing shall be performed or supervised by a competent microbiologist or technician who is experienced and qualified to degree level in microbiology or an equivalent field. The personnel shall have at least two years relevant experience with the testing concerned before being allowed to perform or supervise work covered by the scope of accreditation (EA 2002). If the laboratory includes interpretations of test results in reports, this shall be done by authorised personnel with experience and knowledge in specific application, including, for example, legislative and technological requirements and acceptability criteria. The person filling this position shall have successfully completed at least 20 credit hours in microbiology, public health, food safety or other related topics (A2LA 2001). In small, limited service laboratories with no more than five members of staff where 1-3 tests are performed, the technician performing the tests or supervising the tests shall be trained with demonstrated competence in the tests performed by the laboratory.

Training records shall include all relevant internal and external education and personnel performance verifications. Full documentation of records for the training programme's certifications shall be kept (Garfield 1992). This includes the education certificates relevant to the work, records of on the job training, new techniques, authorisation proofs of competency of the personnel, tests and operating equipment. It also includes satisfaction of the personnel and freedom from undue pressures that could jeopardise the laboratory work.

3.2   Accommodation and environmental conditions

(ISO 17025: clause 5.2)

The accommodation and environmental conditions of the laboratory are important to the performance or effectiveness of personnel and equipment. The quality of culture media and reagents may also be affected by environmental conditions. Temperature, humidity, dust and or sterility influence the quality of results. Samples may be contaminated during analysis leading to incorrect results. The typical laboratory shall be comprised of testing facilities where specific microbiological testing and associated activities are carried out. The ancillary facilities such as entrances, corridors, administration blocks, cloak rooms and toilets, storage rooms and archives shall be physically separated from working areas.

Access to the microbiological laboratory shall be restricted to authorised personnel only. In addition, it is generally considered good practice to have separate locations for sample receipt and storage and sample preparation and examination including incubation. Reference organisms, media, equipment, sterilisation or decontamination, and sterility assessment areas are also required. At a minimum, procedures shall be carried out in a sequential manner using appropriate precautions to ensure sample integrity such as the use of sealed containers, segregate activities in time and space and the use of safety hoods. Highly contaminated samples shall be restricted to selected areas, for example when high levels of pathogens may be encountered for pre-enrichments and selective enrichment transfer areas.

An appropriate environmental monitoring programme shall be devised. The laboratory test area shall be air-conditioned to control humidity and temperature. Work space and test area temperature and humidity shall be monitored. The recommended relative humidity is 45-50% RH and temperature 20-25°C (A2LA 2001). Excessive numbers of environmental bacteria, yeasts and moulds shall be controlled by air systems with filters. Verification and monitoring shall be performed using air sampling devices, air settling plates, surface swabs or other appropriate means. These checks are critical to aerobic plate count, yeast and mould tests. In addition, the laboratory shall have a pest control, and cleaning and sanitising programmes. There shall be a documented cleaning and sanitation programme for laboratory fixtures, equipment and surfaces. It shall take into account the results of environmental monitoring and the possibility of cross-contamination. Measures shall be taken to avoid accumulation of dust, by provision of sufficient storage space, by having minimal paperwork in the laboratory and by prohibiting plants, soils and personal possessions from entering the laboratory work area. Personnel protective gear and clothing appropriate for testing should be worn such as protection for hair, beards and hands. Shoes shall be worn in the microbiological laboratory and removed before leaving the area. Training in the use of safety facilities and labelling chemicals, media and reagents shall be properly conducted and updated. Disposal of any waste suspected to be contaminated with the pathogens shall be decontaminated by autoclaving or disinfection prior to its disposal. The disposal of waste shall conform to national/international environmental or health and safety regulations.

3.3   Test methods and validation of methods

 (ISO 17025: Clause 5.4)

It is recognised that validation of test methods is necessary in analytical laboratories. The use of validated methods is important for an analytical laboratory to show its qualifications and competency. Validation is defined in DIN EN ISO 8402 as 'confirmation by examination and provision of objective evidence that the particular requirement for a specific intended use is fulfilled'. Method validation is needed to 'confirm the fitness for purpose of a particular analytical method'. It demonstrates that 'a defined method protocol is applicable to a specific type of test material and to defined requirements of test items' (EAL 1997). Analytical systems reflect the achievement of analytical results that have an acceptable standard of accuracy. The validation procedure is normally determined by the test laboratory, agreed upon between the customer and laboratory or accepted by the authorities and/or the accreditation bodies.

Method validation is basically distinguished from daily activities such as IQC and QA routines (Thompson 2002). Method validation is carried out once or at relatively infrequent intervals during the working life span of a method. It tells the performance that can be expected from it in future, whereas the IQC tells how the method performs over a period of time. Although analytical (test) methods involve a series of procedures from the receipt of a sample to the production of the final result, validation of microbiological test methods reflects test conditions. Quality assurance is the complete set of measures, which a laboratory must undertake to ensure that it can always achieve high-quality data. Besides the use of validated and/or standardised methods, these measures are effective internal QC/QA procedures including the use of reference materials (RMs), quality control charts, participation in proficiency testing schemes and accreditation. The laboratory shall

document QA/QC procedures, including, but not limited to, media QC, incubation times and temperatures, equipment calibration and maintenance process control and standards for approving/rejecting results. Regardless of the type of method, the scope and the application, laboratories must be able to produce reliable data when performing analytical tests for a client or for regulatory purposes.

The laboratory shall use test methods meeting the needs of the client. Where possible, these methods shall comply with the minimum requirements of international, national and/or regional standards. In addition, if there is no specified method, the laboratory shall use an appropriate method that is traceable to a recognised, validated method. All methods not taken from authoritative, validated sources shall be agreed upon by the laboratory and client, with clearly defined expectations and requirements. The suitability of the method shall be checked and confirmed by comparing with specified requirements typically for the intended use of the method.

Laboratories shall retain validation data on commercial test materials used in the laboratory. These validation data may be obtained through collaborative testing and from validation data submitted by the manufacturers and subjected to third party evaluation (e.g. AOAC). If the validation data are not available or not wholly applicable, the laboratory shall be responsible for completing the validation of the method. If a modified version of a method is required to meet the same specification as the original method, comparisons shall be carried out using replicate tests (EAL 2002). Experimental design and analysis of results must be statistically valid. Even when validation is complete, the laboratory will still need to verify on a regular basis that documented performance characteristics in the method can be met by using spiked samples or reference materials incorporating relevant matrices which are less expensive as compared to a full single laboratory validation.

3.4 Equipment and measurement traceability

(ISO 17025: clause 5.5 & 5.6.2)

According to ISO 17025 and Garfield (1992), equipment relies on good maintenance, services, accurate performance and calibration. Advances in design of digital and microprocessor analytical equipment have led to an improvement in the quality of analytical measurements and increased productivity. However, it is difficult to detect slow changes in day-to-day or even week-to-week operations, and since most methods are made by comparing the response of the sample with the response of a standard, evidence of changes in the absolute response of the instrument tends to be overlooked. A general rule in order to commission, monitor, control and/or avoid the occurrence of errors is to plan a consistent practice of periodic calibration of equipment. The laboratory shall document maintenance schedules and procedures.

Traceability refers to a process whereby a measuring instrument or data can be traced in one or more stages with national or international reference material for the test. The elements of traceability include an unbroken chain of comparisons, measurement uncertainty, documentation, and competence, reference to international standard units and retesting or recalibrating. Traceability is a relatively new term, gaining more and more attention in analytical measurement sciences (EAL 1995). Traceability can be assigned to different aspects related to measurements such as traceability of a result, method, procedure, laboratory, product, material and equipment, as there is no single definition of traceability.

3.4.1 Commissioning of equipment

Commissioning of equipment includes Installation Qualification (IQ) and Operational Qualification (OQ). The IQ requires full documents that the equipment has been installed correctly and that it is suitable for use in the test environment. The OQ is the verification that the measuring system will function according to its operational specifications as detailed by the laboratory. The IQ and OQ shall be performed by the manufacturers or suppliers of the equipment. In addition, the suppliers shall train laboratory personnel who will use the equipment.

3.4.2 Control of equipment

The following equipment shall undergo maintenance and servicing as specified: incubators, ovens, refrigerators, freezers, water baths and vial fillers. Balances, safety cabinets, laminar flow hoods, microscopes, pH metres, glassware and plasticware shall be serviced annually, cleaned and sanitised in each use. Autoclaves shall be serviced semi-annually. Visual checks shall be performed as recommended by the manufacturer. The stills, deionised water/reverse osmosis system filters shall be replaced as recommended by the manufacturer. The laboratory shall have a programme for calibrating and verifying the performance of all critical equipment.

Thermometers:

There are two types of thermometers in the laboratory; working and reference thermometers, scale division 0.2°C and 0.1°C respectively. Working thermometers are routinely used to verify the temperature of the equipment and environment while the reference thermometers are used for calibration checks for working thermometers at an appropriate temperature.

Temperature controls of incubators, water baths, freezers and refrigerators:

Arrange different incubators, water baths, freezers and refrigerators for different purposes. Perform routine controls daily before work starts by using thermometers placed in 25 mL of glycerol. However, the extensive control of accuracy shall be checked every six months for air incubators; water baths for incubation; water baths for tempering medium, refrigerators and freezers.

Control of sterilisation:

Use sterilisation tapes for each load of materials into the oven or autoclave by labelling the date of sterilisation and recording the changes of the sterilisation tapes. The autoclaving time and temperature is guided by the manufacturers of equipment, apparatus, culture media and reagents. The maximum volume of liquid media for sterilisation is 1 L and 200 mL of agar media. Record the material, time of start and removal and change of colour of sterilisation tapes (dry heat and wet sterilisation indicator tapes).

Control of distillation and deioniser equipment:
Check distilled and deionised water for its specific conductivity and microbiology quality on a monthly basis. The requirements are specific conductivity/resistance and

microbiological quality. In the cleaning of distillation units, apparatus and tanks, no coating is tolerated.

Control of balances:

Ensure that balances and reference standard weights are calibrated annually and perform calibration checks on a daily basis prior to using the balance.

Control of volumes:

Diluents in bottles and tubes: randomly check for correctness of the final volumes after sterilisation and document them.

Control of pipettes: check for the volumes dispensed by pipetting up to 10 or 25 mL of distilled water at 25°C into a tarred beaker on balance and take the equivalent weight.

Measuring cylinders: measure the required volume of distilled water into the cylinder tarred on balance and take the equivalent weights.

Control of pH meters:

Perform calibration checks by using at least two points standard buffer solutions prior use. Recommended pH values are 4.00 and 7.00. Take temperature into account when measuring the pH of media, 25°C is usually approaching room temperature. Measure the difference in potentials (mV) between the pH buffer solutions and the sensitivity of the electrode in percent (%). Cleaning and handling of the electrodes is usually done according to the instructions of the manufacturers.

3.4.3 Calibration of equipment

Thermometers and thermocouples: The mercury (Hg) reference thermometers shall be calibrated and recertified every five years. Reference thermocouples shall be calibrated annually.

Weights: Reference weights shall be calibrated and recertified traceable to standard unit every five years.

Balances: Balances shall be calibrated annually.

Timers: Timers shall be calibrated to a national time standard annually.

Volume measuring: Volumetric glassware shall be calibrated gravimetrically annually.

Microscopes: Microscope stage micrometers shall be calibrated at installation.

Hydrometers: Hydrometers shall be calibrated to a reference chemical compound annually.

Autoclaves: Autoclaves shall be calibrated (thermometers or thermocouples) annually.

3.5 Culture media and reagents

(ISO 17025: 2005 Clause 4.6 & 5.5)

The International Committee for Microbiology and Hygiene (ICFMH) has proposed a standardised scheme for the quality control of microbiological media. The scheme proposes the criteria and techniques to test the quality of commercially purchased media (Weenk 1995). However there are no specific guidelines concerning the scope and field of application for each method. The European Community Bureau of Reference has taken an initiative to develop and provide laboratories with standardised reference strains and mixed cultures for QC for microbiological methods and media (Corry *et al*. 1999). The most used technique for quality control procedures for solid media relies on colony counting such as the pour plate and surface spread plate techniques. A widely used method for QC of liquid media is the serial dilution (Most Probable Number – MPN) technique. The positive tubes can easily be determined by eye, by measurement of optical density, by changes in conductance or by streaking on to agars.

3.5.1 Checking of culture media

For the purpose of food microbiology, media are critical materials that shall be verified against national standards – reference cultures and/or certified reference cultures.

Prior to taking new batches of media into use its characteristics on selectivity or recovery against previously used media shall be checked by using quality control samples containing mixed microorganisms. Inoculate replicates of routine samples in a new and old media. Selective media should be tested with positive and negative growth of microorganisms. The following techniques may be applied when checking the quality of a new batch, control using reference samples, control of liquid media: the ecometric technique and the Modified Miles and Misra method.

Control using reference samples: Reference samples should be examined routinely at fixed intervals. Its results should be monitored i.e. by using control charts and detecting trends towards deviations during the early stages and corrective action should be taken promptly.

Control of liquid media: According to NMKL (1994) "culture the test strains until the stationary phase is obtained. Suspend the cultures thoroughly, and make a dilution series in the elective media and in a non-elective medium. It is suitable to dilute until 10^{-12}. Dispense aseptically into sterile tubes, five tubes for each dilution. If sterile micro-titre plates are available, dispense 5 x 0.15 mL from suitable dilutions. Read the results after incubation noting growth or no growth. Express the results as the ratio between the MPN value for the test strain in the selective medium and the MPN value for the organism in the reference medium".

Upon receipt of dehydrated media, records shall be kept including the media name or description, the manufacturer's lot number, the laboratory log number, the date received, the date opened, the date prepared for QC, the expiry date and the discard date. All media shall be identified and traceable to QC for each test.

All batches of media and reagents shall be traceable to autoclave records. Record forms shall contain the date, run number, autoclave number (where appropriate), media and reagents/load, time in autoclave, time at desired temperature, and time out of autoclave. Sterilisation equipment and sterilisation processing cycles shall be validated and

documented. All batches of media and reagents, sterilised by means other than an autoclave, shall be traceable to sterilisation records, which shall document the date, media or reagent, confirmation of heating conditions or filtration and the initials of the responsible person(s).

3.5.2   Handling of culture media and reagents

Storage of dehydrated media and reagents:

The conditions of storage of media and reagents are usually specified by the manufacturers. In general it is recommended to store media and reagents at 25 -30°C in a dark place or under refrigeration and or at freezing temperatures.

Preparation of media and reagents:

Prepare media and reagents according to the instructions of the manufacturers. In addition perform the necessary quality controls including pH of the final prepared media and reagents at 25°C. In the case of agar media, take a small volume and allow it to cool down to 25°C and carefully introduce a pH electrode into cooled agar and measure. Check for sterilisation efficiency by incubating some plates without inoculating samples and there should be no growth (false negative control).

Storage and shelf life:

Prepared media should be stored in the dark in refrigerators i.e. at temperatures between +1°C and +6°C. Some media which contain high concentrations of bile should preferably be stored at least between +10°C and +15°C (NMKL 1994). However the keeping quality varies with the composition of the medium and storage conditions, especially temperature (Appendix 2). Melt solid frozen media in a water bath with boiling water, autoclave or microwave oven. Care should be taken to avoid overheating. Melted media should be used once.

3.5.3   Laboratory grade water

The laboratory's water source shall be tested to ensure that it is inhibitor free, i.e., "microbiologically and chemically suitable water". Documentation of performance of the following tests shall be maintained (Table 2) (A2LA 2001 and Benson *et al*. 1998).

Table 1: Microbiological and chemical requirements of laboratory grade water.

Frequency	Requirement	Limits
Daily	Resistance/conductivity	<1.0 megohms/cm at 25°C
Monthly	Total residual chlorine	<0.01mg/L
	Aerobic plate count	< 1000 colony forming units - cfu/mL
Annually	Heavy metals Cd, Cr, Cu, Ni, Pb, Zn	< 0.05 mg/L for each
	Total heavy metals	< 10 mg/L

Note: Bacteriological suitability must "pass".

3.6   Reference standards and reference materials

(ISO 17025: 2005 Clause 5.6.3)

The laboratory shall have procedures for the safe handling, transport, storage and use of reference standards, reference materials (RM), reference cultures and certified reference cultures (CRC) to prevent contamination or deterioration. Reference standards i.e. reference thermometers and weights shall be used for calibration or verification purposes only. Certified reference cultures shall be traceable to a nationally or internationally recognised type culture collection.

Reference cultures from laboratory sources shall be identified and traceable to standard reference sources. RCs and CRCs shall not be transferred more than five times from the original sources. After the fifth transfer, the laboratory may purchase another culture from a type culture collection or re-identify the culture for key biochemical and physiological characteristics using nationally or internationally recognised reference sources. Alternatively, the type culture may be grown then freeze dried, kept in frozen storage and used periodically thereby extending the length of time after which they must be re-purchased or re-identified. The laboratory shall ensure that their suppliers of CRCs comply with the requirements of ISO/IEC 17025 relevant to testing and calibration laboratories.

3.7   Measurement uncertainty

(ISO 17025: 2005 Clause 5.4.6)

Microbiological tests generally preclude the rigorous, metrologically and statistically valid calculation of uncertainty of measurement. Estimates of uncertainty are based on the repeatability and reproducibility of data, but ideally include bias (e.g. from proficiency testing scheme results). The individual components of uncertainty shall be identified and demonstrated to be under control. Their contribution to the variability of results should be evaluated. Some components such as pipetting, weighing and dilution effects may be readily measured and easily evaluated to demonstrate a negligible contribution to overall uncertainty. Other components like sample stability and sample preparation cannot be measured directly and their contribution cannot be evaluated in a statistical manner but their importance to the variability of results shall also be considered.

The microbiological testing laboratories shall have an understanding of the distributions of organisms within the matrices tested and take this into account when sub-sampling. However, including this component of uncertainty in the estimates is not recommended unless the client's needs dictate or require otherwise. The principal reasons for this are that the uncertainty due to distribution of organisms within the product matrix is not a function of the laboratory's performance and may be unique to the individual samples tested. Test methods shall specify the sample size to be used taking into account poor homogeneity or heterogeneity of samples.

The concept of uncertainty cannot be applied directly to qualitative test results such as those from detection tests or the determination of attributes. Nevertheless, individual sources of variability, i.e. consistency of reagent performance and analyst interpretation, shall be identified and demonstrated to be under control. Additionally, for tests where the limit of detection is an important indication of suitability, the uncertainty associated with the

inoculum used to determine the limit shall be estimated and its significance evaluated. Laboratories shall also be aware of the incidence of false positive and false negative results associated with qualitative tests.

3.8   Handling of sample and analysis

(ISO 17025: 2005 Clause 5.8)

Sampling is not part of the laboratory activity unless it is requested. However where a laboratory is requested to do sampling, it shall ensure that a sampling protocol is prepared and used. The sample should be protected against contamination or growth and changes in the levels of microorganisms. This can be done by using proper and sterile sampling instruments, apparatus, labelling, handling containers and immediate transportation to the laboratory.

A sample is the starting point for assuring analytical work in the laboratory. Samples may be delivered by various means such as by a person who collects the sample, by freight or by courier in containers or packages fresh chilled or at room temperature for dry samples. Samples may or not be accompanied by appropriate documentation to advise or guide the laboratory why it was brought, what analysis is desired and the conditions of storage. These circumstances and conditions need to be documented upon receipt of samples for the reasons that the quality of the test results relies on the conditions of the samples at receipt and requests of testing parameters (Garfield 1992). The primary responsibility for accurate and good record keeping lies with the analyst assigned to examine the sample.

The laboratory shall have procedures that cover the delivery of samples and sample identification. The laboratory's system for the identification of samples shall start with giving a unique and unambiguous sample identification number, name of the client, manufacturer's name or sample source and date of sampling. Identification number or description from client, product description, tests desired and/or methods requested, date of receipt, delivery carrier, sample condition and state.

Samples awaiting test shall be stored under suitable conditions to minimise changes to any microbial population present. Storage conditions shall be defined and recorded. If there is insufficient sample or a sample is in poor condition due to physical deterioration, incorrect temperature, torn packaging or deficient labelling, the laboratory shall consult with the client before deciding whether to test or refuse the sample. In any case, the condition of the sample shall be indicated in a test report. Cross contamination is the most critical issue resulting from broken or leaky packaging.

3.8.1   Choice of methods

Sub-sampling by the laboratory immediately prior to testing is considered as part of the test method. If necessary, divide samples into three portions. The first portion is for analysis, the second portion is to be stored for further work and the last portion is for the client if requested. It shall be performed according to national or international standards, where they exist, or validated in-house methods. Sub-sampling procedures shall take into account an uneven distribution of microorganisms (ISO 6887 and ISO 7218). The laboratory shall use ISO microbiological analytical methods and or national, regional and book methods agreed between the laboratory and the clients. In addition the laboratory should have a written

protocol or procedures for the elaboration and approval of methods. The evaluation of test methods includes elaborating and running it in a laboratory and checking against reference samples and comparative examination with other laboratories during the proficiency testing scheme.

3.8.2 *Sample analysis*

The sample treatment protocol or method should be followed (ISO 6887 and ISO 7218). The diluents to be used shall have a concentration of salt or sugar and pH that is equal to the sample. When making dilutions ensure that pipettes are changed at every step. Inoculation in (tubes and plates) and spreading or mixing of diluted samples should be done within 10 to 20 minutes of dilution. Pour agars at the right temperature, i.e. not exceeding $45\pm1°C$, and mix properly. Ensure that temperature and time requirements during incubation are in accordance to the test method. Read the plates or tubes at the end of the incubation time ±2 hours unless otherwise stated in the method. Carry out confirmatory tests if required by using positive and negative controls and reference organisms if available.

3.9 Assuring quality of test results and reporting

 (ISO 17025: 2005 Clause 5.9 & 5.10)

3.9.1 *Quality of test results*

A key aspect of reliability or validity of results is that they are comparable and traceable, whatever their origin. The reliability of results depends on the precision and trueness. The precision refers to the ability to produce the same results when repeating the test while the trueness refers to the difference between the obtained number of bacteria and the real number. The quality of results reflects the adequacy or inadequacy of a method in terms of the extent to which the method fulfils its requirements or is fit for its particular analysis. Quality is a relative notion, referring to requirements fixed beforehand on the basis of national or international regulations or customer needs. The reliability of analytical data is important as measurement results will be used and or form the basis for decision-making.

3.9.2 *Evaluation of test results*

Selection and counting of the colonies is done in accordance with the requirements of the method. However, the normal range is between 30 and 300 and or from 25 to 250 colonies grown into the plates. The outcomes of counts are calculated against the dilution factor. Control of the precision of counting colonies should be verified by repeating either by own counting on the same plate with an accuracy of 7.7%, and or between different personnel with an accuracy of 18.2% (NMKL 1994). The MPN results are obtained from the MPN table on the basis of the results on positive tubes and series of tubes used i.e. three or five tubes MPN series (Appendix 3).

The head of the laboratory is responsible for the correctness and trueness of the analytical results to be signed and dispatched. If the results deviate significantly from expectations or guide values, they must be verified and or rejected. However if the results are rejected analysis may be repeated or a new sample requested from the client. When evaluating the test results take into account the available data of a sample.

3.9.3   Reporting the results (test reports)

(ISO 17025: 2005 Clause 5.10)

The microbiological analytical results are reported as cfu/g or mL for the colony count method and or MPN/g or mL (3 or 5) tubes MPN. If the result of the enumeration is negative, it shall be reported as "not detected for a defined unit" or "less than the detection limit for a defined unit". The result shall not be given as "zero for a defined unit" unless it is a regulatory requirement. Qualitative test results shall be reported as "detected/not detected in a defined quantity or volume". They may also be expressed as "less than a specified number of organisms for a defined unit" or more than, where the specified number of organisms exceeds the detection limit of the method and this has been agreed with the client. The client shall be notified of any factors that have affected or may potentially affect the integrity of results and shall be informed of any interpreted data or evaluations that are made.

3.9.4   Documentation of analytical work and internal quality control

In analytical quality assurance it is essential that all data generated during sample handling, analysis and results are documented in such a way that a determination can be repeated in the same way as it was originally done. This makes it easy to trace back results when needed for the purpose of verification, evaluation or audit. Records are required that are signed off against each record of activity being performed in a laboratory. If there is any change in the data it must be crossed and not erased.

Internal quality control consists of all the procedures undertaken by a laboratory for the continuous evaluation of its work. The main objective is to ensure the consistency of results from time-to-time and their conformity with defined criteria. The programme of periodic checks is necessary to demonstrate that variability, i.e. between analysts and between equipment and materials, is under control. The programme may involve the use of spiked samples, reference materials including proficiency testing scheme materials, replicate testing, and replicate evaluation of test results. The interval between these checks will be influenced by the number of actual tests. It is important, where possible, that tests incorporate controls to monitor performance. According to Taverniers *et al.* (2004), between-analyst within laboratory precision is useful in the assessment of analyst performance. The precision shows how closely two or more analysts in the laboratory, by using the same method, can check and compare to each other. Their results can be monitored by quality control charts.

Quality control charts are charts in which the trends of quality control analytical results are continuously filled in at regular intervals. They consist of two types of limits the warning and action. For example the warning limits correspond to ±2 standard deviations from the mean value. On the other hand, the action limits are set at ±3 standard deviations from the mean value. Control charts are useful tools in keeping analytical results. When the test results or data exceeds the control limits, this indicates that an error may have occurred. The greater the deviation from the mean value, the more likely it is that a systematic error is present. The laboratory should take prompt action to trace any cause of the error and take appropriate corrective actions for the noticed error.

3.10 Proficiency testing

(ISO 17025: clause 5.9b)

Proficiency testing refers to examining series of identical sample materials by a number of laboratories independently of one another. The participating laboratories are required to follow instructions and perform analysis by using their routine test methods and procedures, then send test results to the organiser for evaluation and statistical calculations which are compiled and sent to all participating laboratories.

The inter-laboratory proficiency testing scheme is the periodical assessment of the competency or the analytical performance of individual participating laboratories (Eurachem guide 2000). The primary aim of proficiency testing is to provide a quality assurance tool for individual laboratories to enable them to compare their performance with similar laboratories. If there are any non-conformances, the laboratories should take necessary remedial actions. Proficiency testing a powerful tool to help a laboratory to demonstrate its competence to an accreditation body or other third party. In addition, proficiency testing enables laboratories to monitor their tests over time. Long-term trends can be identified and necessary corrective action shall be considered. Improvement and maintenance of the quality in the laboratory can be important for accredited laboratories, or those seeking accreditation, from regular participation in appropriate proficiency testing schemes. The results of participating laboratories in the inter-laboratory comparisons are taken into account by the accreditation bodies. They demonstrate the competence of the laboratory to carry out the test method of interest (EA 2001). Laboratory proficiency testing can be achieved through several ways including the following:

Qualitative schemes: for example where laboratories are required to identify a component of a test item.

Single item testing: Where one item is sent to a number of laboratories sequentially and returned to the organiser at intervals.

Continuous schemes: Where laboratories are provided with test items at regular intervals on a continuing basis.

Validation of methods: For validation of methods, inter-laboratory comparisons are used as a means for determining the key performance characteristics such as reproducibility, comparability, robustness, measurement uncertainty. The results from such comparison exercises can be used to determine laboratory competence by reference to the performance criteria. Performance characteristics of test methods under comparable conditions of limiting values or robust mean are useful targets in such inter-laboratory comparisons.

Self-assessment of a laboratory's performance in a test: When a laboratory reviews its quality management system, inter-laboratory comparisons are one of the tools used to evaluate the laboratory's performance.

Bilateral proficiency test (Check/QC sample test): The laboratory receives a test item with accurately determined characteristics, which are to be tested in the frame of an accreditation procedure. The test item is given either by the assessor or provided by a third party.

3.11 Internal audit

(ISO 17025: 2005 Clause 4.14)

The laboratory shall carry out internal audits at regular intervals to ensure that its quality system is fully implemented in practice. The internal audit checks on whether or not the requirements stated in the laboratory quality manual and its related documents are applied at all levels of work by the laboratory. There shall be scheduled plans and checklists made to include all elements in the quality system to ensure that all are checked at least once a year. The non-compliances that are found during the internal audits provide valuable information for improvements in management and the quality system. The audit programme combines both the horizontal and vertical audits (EAL 1996).

The horizontal audit is a detailed check of quality system elements through a total range of testing activities covered by the laboratory including personnel training, equipment and test methods. The vertical audit is a detailed check that the entire quality system associated with the tests is implemented in a specific test item as selected randomly. It includes the sample handling, personnel involved, equipment calibration and performance, procedures and test methods used, and quality control requirements. Monitoring of environmental conditions during testing, test records and reporting of results and data storage. In addition unscheduled audits may be conducted as necessary due to either internal or external non-conformances or verification on agreed corrective actions. The records of internal audits shall be documented and kept.

3.12 Management reviews

(ISO 17025: 2005 Clause 4.15)

The laboratory shall carry out a formal management reviews meeting at least once per year and when necessary an informal management meeting shall be held. The participants of the management review meeting include all senior personnel with influence in the implementation of the quality system. It shall be chaired by the laboratory manager or the overall management representative. The secretary shall be the quality manager as the one responsible for the overall laboratory quality system.

The agenda for this meeting shall include matters arising from the previous meeting, assessors and surveillance reports, internal and external audits, internal and external proficiency testing results. The agenda shall also include complaints, needs for improvements in the quality system, vacancies, resource requirements, personnel training, and development projects (EAL 1996). The findings and actions arising shall be recorded and their corrective actions shall be carried out within an agreed timeframes.

4   RESULTS

4.1   Guideline requirements for internal QC /QA

4.1.1   General requirements

The purpose of accreditation of a laboratory is to demonstrate its competence in performing defined tasks. The basis of reliability of the test results is the recognition of guidelines on IQC/QA routines with effective planning, monitoring and verification programmes. Guidelines for test methods, equipment, culture media and reagents, environmental conditions, sample, quality of test results and personnel need to be documented and implemented (Bolton 1998).

The quality of microbiological test results is dependent on the quality of the sample tested. The laboratory must properly check for the correct documentation and conditions of the sample prior to accepting it. A sample has to be stored at the correct conditions before testing to prevent or minimise changes in the microbiological status. Quality control in culture media must be in place before being released to be used for culturing bacteria. Essential checks include productivity, selectivity, colonial morphology, sterility, pH and appearance. Commercial culture media quality control guidelines are provided by the manufacturer or test methods. In addition, it is important for a laboratory to use traceable reference cultures. Physical or visual QC checks for the calibration and performance on mass and volume measurements are important for correct sample preparation. Any error resulting at this stage affects the quality of the results. Daily monitoring checks and regular cleaning and maintenance programmes are necessary to ensure the satisfactory performance of equipment.

The performance and validation on test methods/ and procedures can be achieved by using combinations of duplicate and replicate samples, reference materials and or spiked samples. The use of duplicate samples is applicable to the quantitative methods. It has to be done by at least two analysts to give an indication of performance and consistency of results. Reference materials may be used in qualitative detection methods. The frequency of QC testing is dependent on the number of samples tested, personnel involved and or frequency of use of the test method. The final test report must be thoroughly checked so as to meet the specifications, national or international standards. The accuracy of results is implied by its precision and trueness. The precision as referred to measurement uncertainty estimates in terms of repeatability and reproducibility precision measures. The trueness refers to systematic deviation of measured results from a true result (Taverniers *et al*. 2004).

4.1.2   Monitoring frequency and documentation

The internal QC and QA have been listed for the controls of equipment, culture media and reagents, samples, test methods and procedures, test results, proficiency testing, laboratory house keeping and sterility, personnel, internal audits and management reviews (Table 2). In addition, various forms have been formulated that can be used as working tools in the documentation of activities and data gathering in the laboratory (Appendix 3).

Table 2: Suggested internal quality control and assurance guideline requirements for a food microbiology laboratory (ISO 17025, A2LA 2001 and EA 2002).

No	Internal QC / QA	Requirements	Frequency or guide values	Verification forms /documents
1	**Control of equipment (maintenance, monitoring, verification and calibration)**	Reference thermometers, working thermometer and autoclave maxima thermometer calibration and calibration checks	Calibration-every five years Annually Monthly	Form 03 Calibration certificate
		Temperature controls on incubators, refrigerators, freezers and baths	Daily	Form 03
		Autoclaves and oven sterilisation-sterility checks and cycle verification by tapes	Every cycle or load	Form 02 & 08
		Water still and deioniser maintenance – conductivity microorganism counts	Weekly Weekly	Report/manual Form 09
		Balances - calibration checks Calibration	Daily Annually	Form 07 Calibration certificate
		Volume measurements – pipettes and cylinders	In each adjustment Annual calibrations	Form 03 Calibration certificate
		pH meters	Daily	Form 06
		Hydrometer calibration	Annually	Calibration certificate
		Timers calibration	Annually	Calibration certificate
2	**Control of media and reagents**	New media batch - positive and negative growth of microorganisms	Every batch received	Form 09
		Media QC – pH checks	Every preparation	Form 04
		Positive and negative quality checks on agar	Every test	Form 09
		Storage temperature for media	25-30°C	Form 03
	Laboratory grade water	Water resistance at 25°C	Monthly	Test report
		Total residual chlorine in water	Monthly	Test report
		Microbiological quality - TPC	Daily	Form 09
		Heavy metals (Cd, Cr, Cu, Ni, Pb, & Zn	Annually	Test certificate
3	**Control of sample**	Sampling and receipt condition checks	Every sample	Form 01
		T & t on analysis and incubation	Follow 100%	Test method
4	**Test methods and /procedures internal QC**	Replicate samples/duplicate	Daily/monthly	Form 09
		Reference materials and or spiked samples	Monthly	Form 09
5	**Control of test results**	Evaluation of test results	Every test results	Form 09
		Documentation of sample data and records	Every step	Form 09
6	**Proficiency testing/ Inter-laboratory comparisons**	Qualitative test schemes	New methods or test parameter	PT report/ form 09
		Single item testing	Organiser/client	PT report/form 09
		Continuous testing scheme	Monthly	Form 09
		Validation of test methods	New methods	Report
		Laboratory self-assessment in performance in tests	Daily	Form 09
		Bilateral proficiency (check sample) test	Organiser/client	PT report/ form 09
		Blind test item	Organiser/client	Report /form 09
7	**House keeping and sterility**	Relative humidity (RH) Room temperature (RT)	45 to 50% 20 to 25°C	Form 03 & 05
		Environment air and swabs - sterility checks	Weekly	Form 09

		Cleaning Sanitation	Daily Weekly	Cleaning record
8	**Personnel**	Competency training records for technical personnel	New techniques	Form 13
9	**Internal audit**	Audit whole quality system	Annually	Form 11 & 12
10	**Management review**	Formal management meetings	Annually	Form 10

4.2 Microbiological analysis results

The quantitative analyses of 25 replicate samples total plate count showed that *Staphylococcus aureus* and coliforms were positive in all replicate samples (Table 3). *Enterobacteriaceae* and *E. coli* were only positive in the control samples (Table 4). In total plate counts the standard deviation of 25 replicate samples was 0.26 and 0.23 for five replicate sample means. The standard deviation for *Staphylococcus aureus* counts observed for 25 replicate samples was 0.31 whereas the sample mean standard deviation was 0.28. The standard deviations for replicate samples of coliforms MPNs were 0.36 and 0.35 for sample means. In the control sample standard deviation was about 0.38 for total plate count and 0.35 for *Enterobacteriaceae,* 0.51 coliforms MPN, 0.15 *Staphylococcus aureus* counts and 0.56 for *E. coli* MPN. The mean values (mv) and standard deviations (s) (mv±2s & mv ±3s) of fish and control samples analysed were used to make control charts (Figures 1 to 4).

Table 3: Mean values and standard deviations from analyses of fish samples.

	TPC (log cfu/g)	St. Aureus (log cfu/g)	Coliform (log MPN/g)
$mv_1 \pm s_1$ (n = 25)	4.24 ± 0.26	3.81±0.31	0.18±0.36
$mv_2 \pm s_2$ (n =5)	4.29±0.23	3.82±0.28	0.17±0.35

[1] Values from 25 replicate samples

[2] Values from replicate sample means

Table 4: Mean values and standard deviations from analyses of QC samples.

mv ± s	TPC (log cfu/g)	*Enterobacteriaceae* (log cfu/g)	*St. Aureus*(log cfu/g)	Coliforms(log MPN/g)	*E. Coli* (log MPN/g)
(n = 6)	6.43±0.38	5.30±0.35	4.69±0.15	4.54±0.51	3.75±0.56

4.2.1 *Detection of Salmonella and Listeria monocytogenes*

The qualitative analyses results for the detection of *Salmonella* and *Listeria monocytogenes* from spiked samples were all positive (+ve) (Table 5).

Table 5: Detection results of spiked *Salmonella* and *Listeria monocytogenes* from fish samples.

a). Detection of *Salmonella*										
Enrichments	**Selective agar**		**TSI**				**LIA**			
			Slant	**Butt**	**Gas**	**H₂S**	**Slant**	**Butt**	**Gas**	**H₂S**
1ˢᵗ LB	HE	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	XLD	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2ⁿᵈ RV	BS	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
1ˢᵗ LB	HE	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
	XLD	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2ⁿᵈTT	BS	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

b). Detection of *Listeria monocytogenes*						
Enrichment	**Fraser**	**Mox**	**TSA-YE**	**Catalase**	**Gram**	**Motility (BHI)**
			5 colonies	5 colonies	5 colonies	5 colonies
UVM	Black (+ve)	Black (+ve)	Positive	Positive	Positive	Positive

4.2.2 *Total plate count*

In the control charts for total plate count for 25 replicates and control samples (Figure 1), all replicates were within the warning limits (mv±2s), however out of 25 replicate samples 12 replicates were above the mean values (mv + 2s) and 11 below (mv-2s), (Figure 1a). Two out of five replicate sample means were in the line with the mean value whereas one replicate was below the mean value and two above the mean value (Figure 1b). Out of six replicates four were below the mean value and two above the mean value (Figure 1c).

(a)
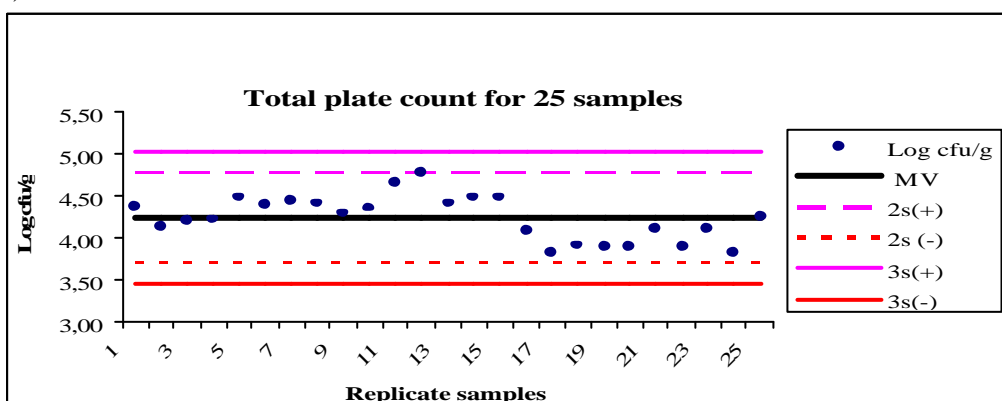
(b)
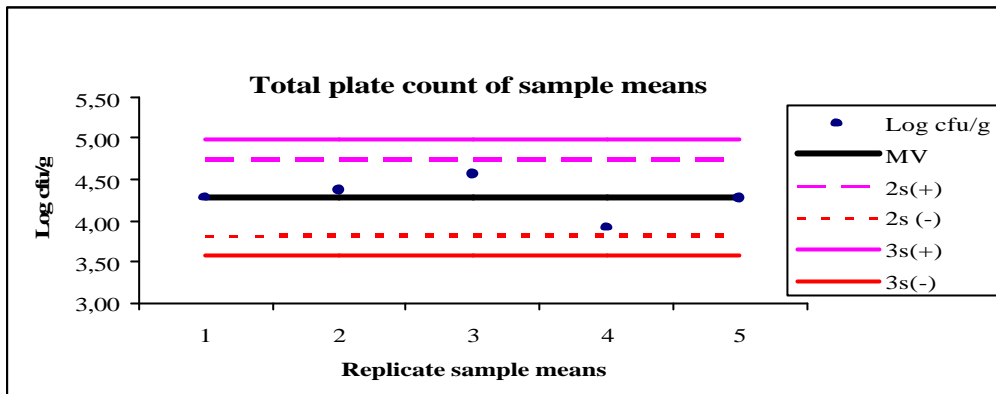
(c)

Figure 1: Control charts for total plate counts at 30°C for 48 hours. (a) Twenty five replicate samples, (b) five replicate sample means and (c) six replicates of two QC samples.

4.2.3 *Staphylococcus aureus counts*

The control charts for *Staphylococcus aureus* counts all replicates were within the warning limits (mv±2s), (Figure 2). Fourteen replicate samples out of 25 were within the upper warning limit and 11 were in the lower warning limit (Figure 2a). All five replicate sample means were within the warning limits (mv±2s) (Figure 2b). Out of six replicate control samples five were found within the warning limits and one in the margin of the lower warning limit (Figure 2c).
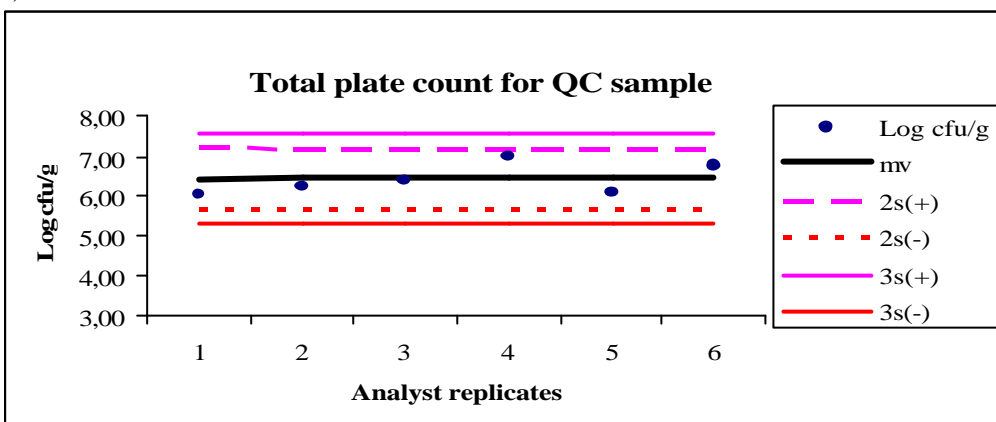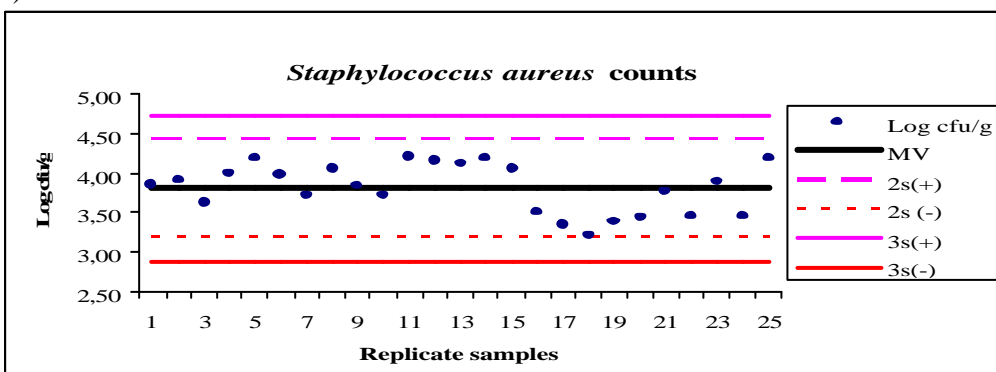
(a)

(b)
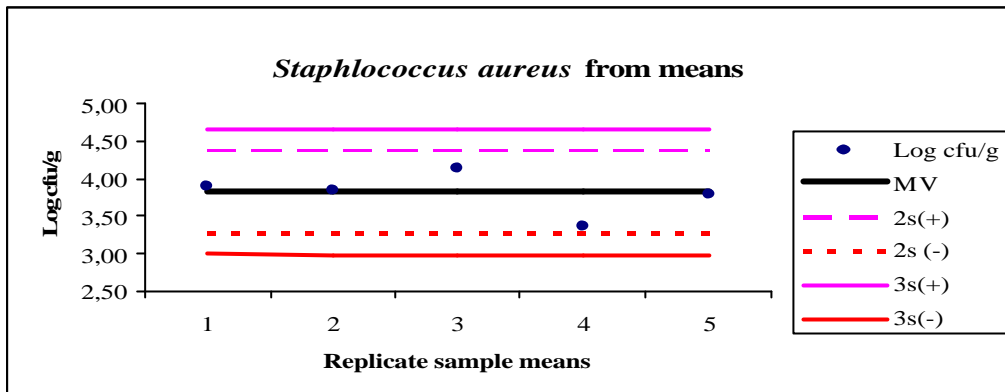
(c)
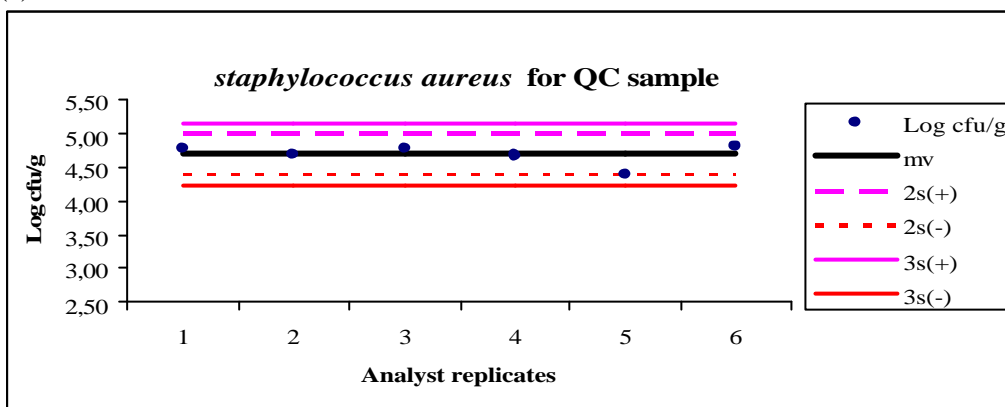
Figure 2: Control charts for *Staphylococcus aureus* counts at 35°C for 72 hours. (a) Twenty five replicate samples, (b) five replicate sample means and (c) six replicates of two QC samples.

4.2.4   Coliforms determination

A control chart for coliforms shows that all replicate samples were within warning limits (Figure 3). Fourteen replicates were within the upper warning limit and 11 in the lower warning limit (Figure 3a). Two out of five replicate sample means were within the lower warning limit and three in the upper warning limit (Figure 3b). Two replicate numbers were within the lower warning limit and four in the upper warning limit (Figure 3c).

(a)
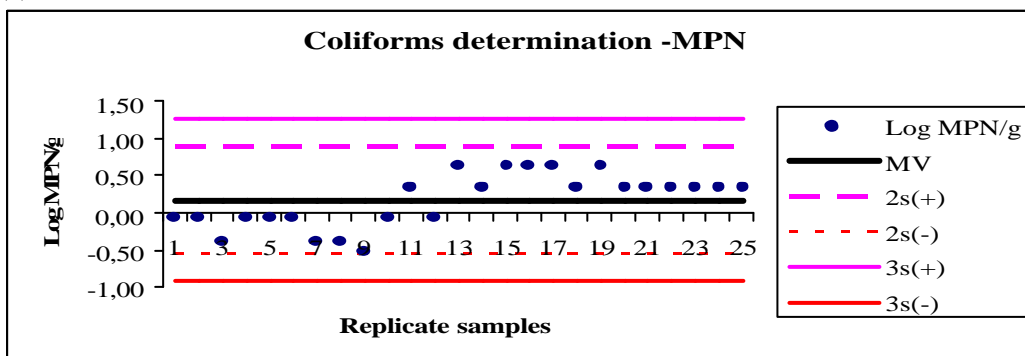
(b)

(c)
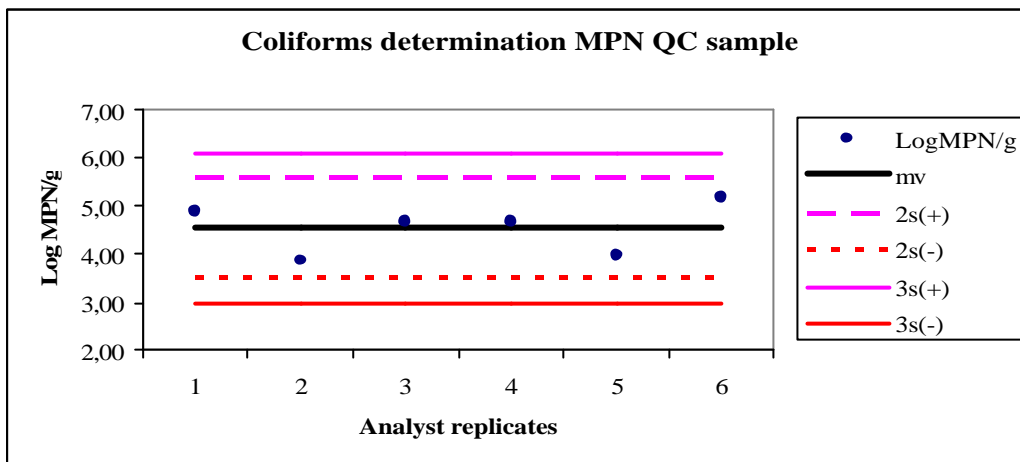
Figure 3: Control charts for coliform determination by most probable number (MPN/g) at 35°C for 48 hours. (a) Twenty five replicate samples, (b) five replicate sample means and (c) six replicates of two QC samples.

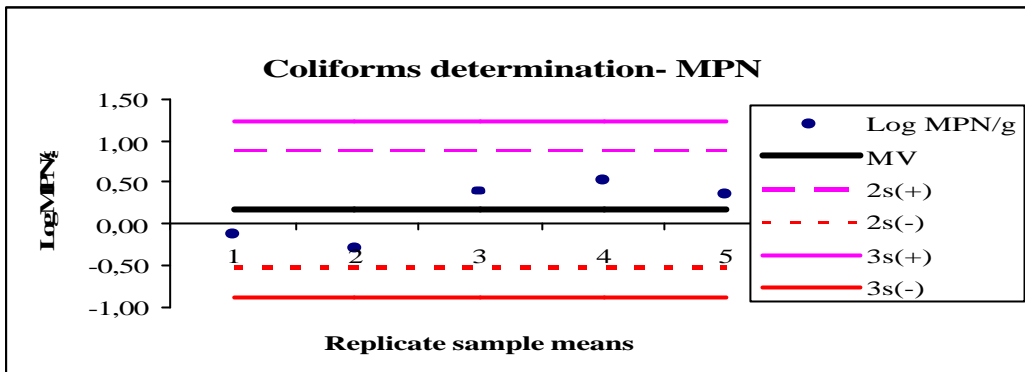### 4.2.5   Enterobacteriaceae and E. coli determination

For the control chart of *Enterobacteriaceae* counts all replicate QC samples were within the warning limits, four in the upper warning limit and two in the lower limit (Figure 4a). All replicate numbers were within the warning limits for the control chart of *E. coli* (Figure 4b).

(a)
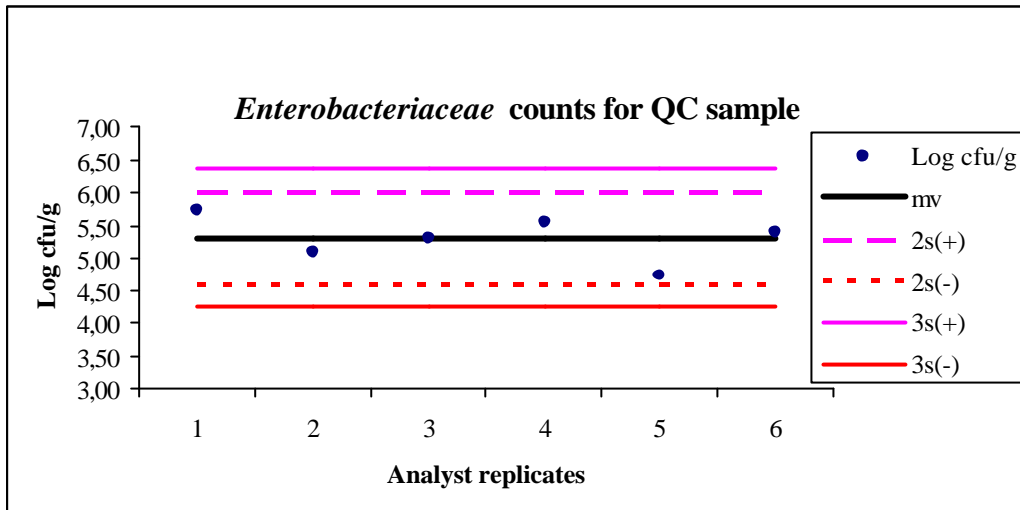
(b)
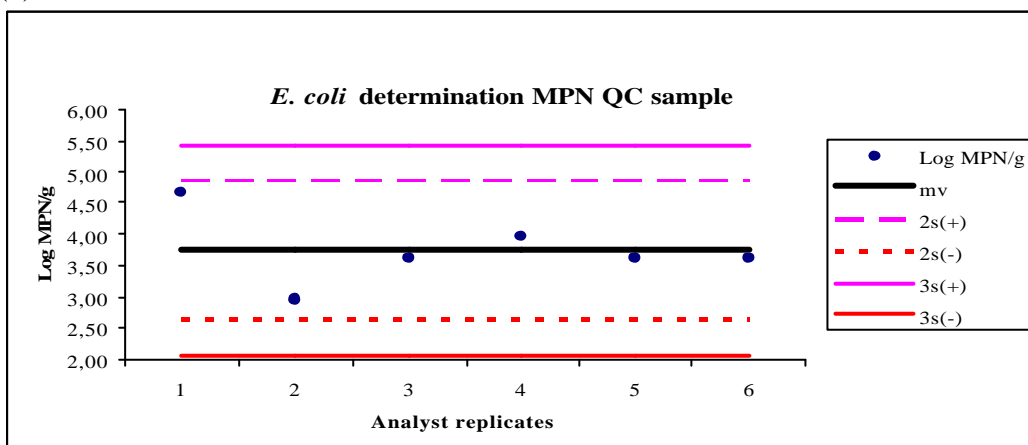
Figure 4: Control charts: (a) *Enterobacteriaceae* count at 35°C for 24 hours and (b) *E. coli* determination by most probable number (MPN) at 45±0.2°C for 24 hours.

5   DISCUSSION

The requirements set out in the ISO 17025 guidelines on internal QC and QA routines have been listed, set up and validated for a microbiology laboratory. They include control of equipment, test methods, culture media and reagents, environmental conditions, sample, test results and personnel. Performance of individual personnel is of prime importance. One way of monitoring performance is by using reference standard samples.

Good maintenance, services, calibration and monitoring performance of equipment play an important role in the results. Daily temperature monitoring, balance calibration and pre-

checks on correct performance of the equipment in the laboratory become routine prior to starting work start.

The culture media and reagents used in analyses in food microbiology are considered to be critical materials that should be verified against national standards – reference and/or certified reference cultures. They should be monitored and checked before being used. Check for pH and sterility of media. The results of positive and negative tests are an indication of the quality of media and efficiency of sterilising equipment.

Aseptic handling of samples during storage and analysis is required in order to avoid growth and or contamination of microorganisms. This requirement might be important since results of samples are considered to be true representatives of the main product. Use of test methods that are correctly validated and running in a laboratory play a role in the quality of the results.

For assuring the quality of the test and calibration results, the associated data should be recorded in such a way that trends are detectable and traceable. Where practicable the statistic techniques shall be applied in reviewing the results (ISO 17025 2005). Often the control charts are used to follow trends in analyses of homogenous reference samples in the laboratory. According to Taverniers *et al*. (2004), between-analyst within laboratory precision is useful in assessing analyst performance. The results enable comparisons for the performance of several analysts in the laboratory using the same method. The trends of test results can be monitored by the control charts (EA 2001).

The results from the samples analysed indicated significant standard deviation where replicate analyses were found either below or above the mean values within warning limits (mv±2s) in the control charts. The standard deviation for coliforms and *E. coli* were 0.35 and 0.36 compared to 0.28 of *Staphylococcus aureus* and 0.23 for plate count. In the control samples the standard deviation for coliforms was 0.51 and 0.56 for *E. coli,* and were very big compared to other parameters. This might be due to the high precision of the MPN procedures as compared to colony count methods. The detection limit of the MPN method is 10 organisms (Peeler *et al*. 1992). The detection limits for the colony count method starts from 25 and or 30 visible countable colonies grown in the plates. In the total plate counts, out of 25 replicate samples 12 replicates were above the mean values (mv + 2s) and 11 below (mv-2s) (Figure1a). There was an increase in levels of counts in total plate count and *Staphylococcus aureus* count from run one to three and a sharp decrease in the two last run samples after storage (Appendix 4). One reason, which contributed to the deviation and sample behaviour, might be the heterogeneity of the samples resulting from poor distribution of microorganisms or preparation. Spiking with *Staphylococcus aureus* was done after mincing and mixed by hand using a spatula before frozen storage. It was possible that some microorganisms were dead during storage where some of the samples were stored up to nine days. The case was not applied the same to the qualitative spiked samples with *Salmonella* and *Listeria monocytogenes* where spiking was done on the same day of analysis before weighing and all results for replicates samples analysed were positive. Also few data were used to construct control charts, which might also have contributed to this deviation.

According to Garfield (1991), a control chart is constructed by analysing at least 10 duplicate test samples (20 analyses). This rule was not used in this experiment. When duplicate tests are used in analysis the accuracy-precision measure may increase. Use of

control samples containing defined ranges of guide values from lowest to highest detectable limits is better. When performing analysis in control samples, analysts expect to obtain results within the guide values. An unexpected systematic errors might be found when some data go outside control limits. The alerts should be attended immediately to correct or eliminated the errors.

The internal QC and QA monitoring routines for equipment and analyses has been familiarised and practiced including proficiency testing and test method validation. Knowledge of control charts, which seemed to be one of the simple techniques for monitoring trends in the quality of analytical data have been understood. Generally this work has been successful and has come out with various guidelines and verification forms, which are suggested to be applied by the National Fish Quality Control Laboratory.

6   CONCLUSIONS AND RECOMMENDATIONS

It is concluded that implementation of the IQC and QA routines will benefit the NFQCL in its progress towards seeking accreditation in the near future.

To obtain analytical measurements and results that are fit for the purpose, samples must be collected and handled correctly. The measurement methods must be validated and effectively controlled, suitable calibration and control standards must be used and all of the personnel involved in the analytical activities must be qualified and competently trained.

However compliance of accredited laboratories depends on good planning and validating internal quality control and quality assurance routines for its competency in performing tests.

The application of control charts in quality controls and quality assurance practices in food microbiology laboratories needs a correct approach starting with the preparation and handling of the sample. Sample matrix or characteristics guide values/limits of interest must be pre-defined by competent personnel.

The conformity assessment is an important component to the world economy due to global increases in trade across borders. For instance in the international trade in food commodities including fish and fish products there have been ever-increasing demands for high value commodities and safety to consumers. Many products require testing and supporting technical documents that include test data for conformance with specifications, compliance with safety, or other regulations before they can be put on the market.

Traceability in laboratories practices is another area of concern. Indirect application of HACCP principles is necessary to ensure high quality of laboratory data, which represents the quality and safety of the products tested. Finally, results give product safety assurance to the ultimate consumers. In Tanzania, neighbouring countries, regional standards and accreditation bodies are required to be harmonised and prepare guidelines for laboratories quality assurance and accreditation.

ACKNOWLEDGEMENTS

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APPENDIX 1: MICROBIOLOGICAL ANALYSIS PROCEDURES

Examination of Microorganism: -Total plate count

Sample preparation, serial dilutions, inoculation, pour plating and incubation at 30°C for 48 hours.

Blend (solid sample), weigh 25g, add 225mL diluent and homogenise by stomacher

↓

Inoculate in replicate plates 10^{-1}, 10^{-2} 10^{-3}, to 10^{-n} (optional)

↓

Pour approximately 10 to 15mL molten PCA, mix well and leave to solidify

↓

Incubate at 30°C for 48 hours and read
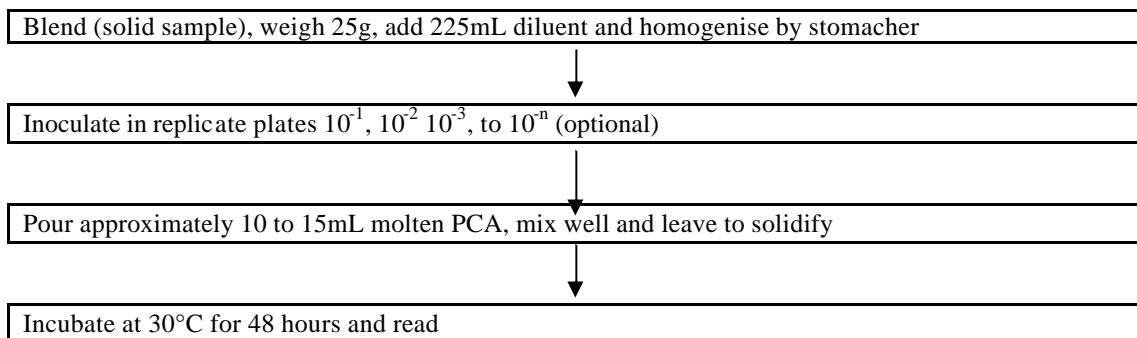
Enumeration of coliforms by MPN method

A: Preparation and enrichment into lauryl sulphate tryptose (LST) broth

Blend (solid sample), weigh 25g, add 225mLs in diluent and homogenise by stomacher

↓

Prepared LST tubes 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, to 10^{-n} (optional)

B: Presumptive test for coliforms

↓
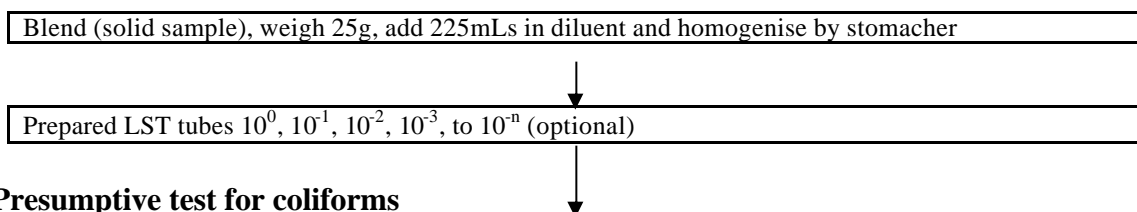
Inoculate 10mLs(DS), and 1mL from each dilution in LST tubes and incubate at 35°C for 48 hours
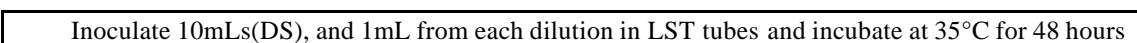
C: Confirmation tests for coliforms

↓

Subculture positive tubes in EC and BGB tubes (incubate at 35°C-48hrs incubator, 44.5°C 24hrs water bath respectively

↓

Read after incubation time
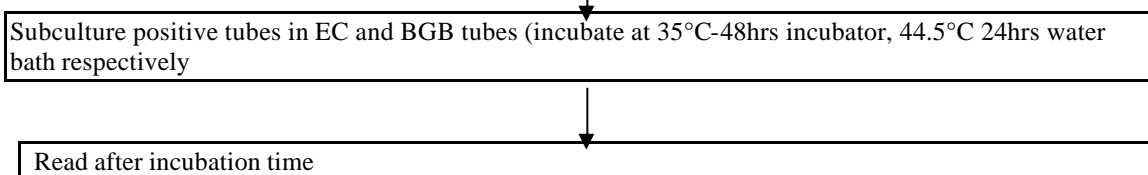
Examination of Enterobacteriaceae

Blend (solid sample), weigh 25g, add 225mL diluent and homogenise by stomacher

↓

Inoculate in replicate plates 10^{-1}, 10^{-2} 10^{-3}, to and 10^{-n} (optional)

↓

Pour approximately 10 to 15mL molten VRBGA, mix well and leave to solidify, pour overlay about 10mL VRBGA

Incubate at 35°C for 24 hours and read

Examination *of* Staphlococcus aureus

| Blend (solid samples), weigh 25mL, add 225g of diluent and homogenise stomacher |

↓

| Homogenise with stomacher |

↓

| Inoculate 2 x 0.5mL into 110 (with 25mL BHI & EY) and spread by spreader rods |

↓

| Incubate at 35°C for 72hrs |

Detection of Listeria monocytogenes

A: Preparation and primary enrichment into UVM –University of Vermont broth

| Blend (solid sample), Weigh 25g and add 225mLs UVM |

↓

| Homogenise by stomacher for 2 min, incubate for 24 ± 2hrs at 30°C (in bags-CO_2) |
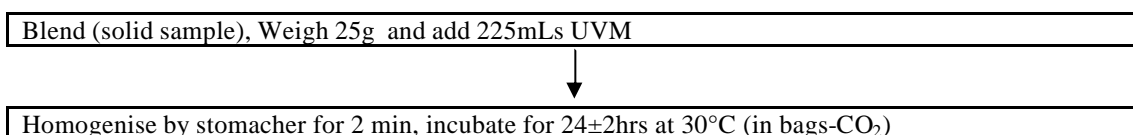
B: Secondary enrichment into Fraser medium

| Transfer 0.1mL(UVM broth) into Fraser with $0.1Fe(NH_4)_3$; incubate for 24/40/48hrs at 35/37°C |
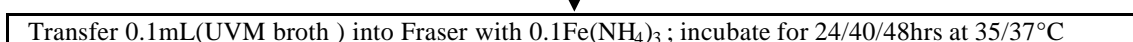
C: Selective plating into Modified Oxford medium (MOX)

| Streak positive tubes in MOX plate incubate for 48hrs at 35°C |
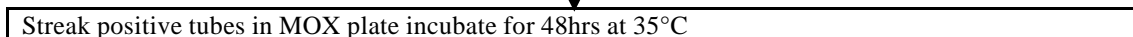
D: Purify *L. monocytogenes* in Trypticase soy agar-yeast extract (TSA-YE)

| Streak positive plates onto TSA & YE plate (5-6 colonies in sections of one plate) |
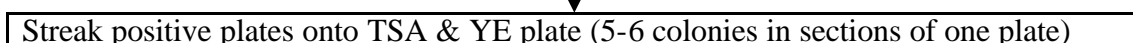
E: Identification of *L. monocytogenes* isolated colonies – Biochemical & Microscopic examin.

| Catalase test (by H_2O_2) |

↓

| Gram staining/colouring |

↓

| Inoculate isolated colony in 5mL Brain heart infusion (BHI) broth, incubate at 20 to 25°C for 24 ±2hours. |

↓

| Microscopic examination- by oil immersion objective to permit a characteristic of slow tumbling motality and short-rod morphology of culture |
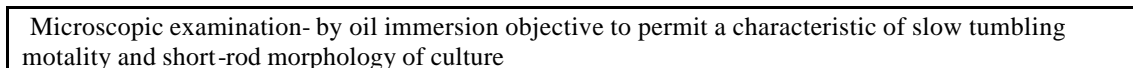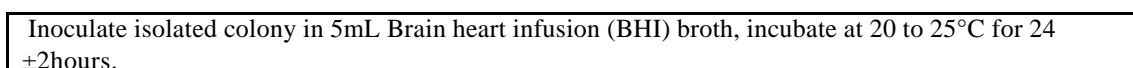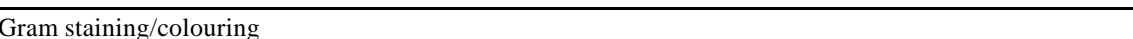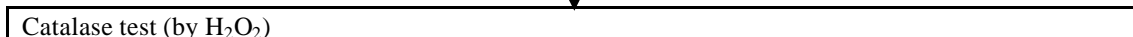
Note:

Use BHI cultures to inoculate all biochemical, the motility medium. Biochemical reaction in TSI (a/a acid slant/acid butt), catalase test (+ve), O/F glucose (+ve), gram reaction (+ve), tumbling motality (+ve), umbrella motility (+ve), Methyl red- Voges Proskauer (MR-VP) - (+ve), Grow and hydrolyse in Bile esculin (+ve), Urea (-ve), oxidase (-ve), etc.

Detection of Salmonella

A: Preparation of sample (pre-enrichment)

> Blend (option to solid samples only), weigh 25g and add 225mLs of Lactose broth (LB) (contaminated or spiked sample in Selenite Cystine (SC) and Tetrathionate Broth TB

> Homogenise by stomach for 2minutes, leave to settle

> Check pH (6.8 ± 0.2) and adjust by acid or base as required and incubate for18 to 24 hrs at 35 or 37°C

B: Isolation of Salmonella (Selective enrichment broth)

> Transfer 0.1mL into 10mLRV and 1mL into 10mL TT and mix by vortex mixer

> Incubate: RV tubes at 42 ± 0.2°C for 24hrs (water bath) and TT tubes at 35 ± 0.5°C for 24hrs (add 0.1 mL brilliant green and 0.2 mL KI solutions in TT)

C: Isolation of salmonella (Selective plating media)

> Mix (RV and TT) tubes thoroughly and streak each onto BSA/BGA, XLD and HE; incubate at 35/37°C for 18 to 24hrs or up 48 hours for BSA plates

D: Biochemical media

> Streak positive plates into LIA & TSI (store plates at 5-8°C), incubate 35°C for 24hrs

E: Identification of Salmonella (Urease test- conventiona/rapid)

> Aseptically(needle) inoculate positive TSI into urea tubes; incubate 35/37°C for 24±2hrs or transfer 3mm loop into urea tube37±0.5°C for water bath parallel with a negative control.

Note:
ISO methods: pre-enrichment buffered peptone water, selective enrichment SC/SBG (35/37°C, RV/TT (42-3°C). Comparative efficiency of subculturing smaller (0.1 mL) or larger 2 mL and 10 mL; there is no significant difference in method sensitivity after pre-enrichment cultures are incubated overnight at 35 to 37°C for 18 to 24 hours. Highly contaminated samples with *salmonella* are recommended to be inoculated directly to selective enrichment. BGA plating media is compulsory when using ISO methods. The separation of salmonella from non-salmonella bacteria is obtained through the additions of varies dyes, bile salts and other selective surfactants agents into the agar media and ability to produce H_2S. Colonies appearance: HEA (blue-green to blue); BSA (green); XLD (pink).

The TSI and LIA provide a preliminary screening of suspect cultures of salmonella. TSI: Measures production of H_2S and fermentation of sugars; slant red alkaline and yellow acid butt. LIA: Monitors production of H_2S and lysine decarboxylation;

Most salmonella are urease-negative (no change). False-positive (purple red). Retain suspect positive cultures for further testing.

Table 6: MPN Tables (modified)

MPN WITH THREE TUBES, 1g, 0,1g and 0,01g			
NUMBER OF POSITIVE TUBES			
1g	0,1g	0,01g	MPN/g
0	0	0	<0,3
0	1	0	0,3
1	0	0	0,4
1	0	1	0,7
1	1	0	0,7
1	2	0	1,1
2	0	0	0,9
2	0	1	1,4
2	1	0	1,5
2	1	1	2,0
2	2	0	2,1
3	0	0	2,3
3	0	1	3,9
3	1	0	4,3
3	1	1	7,5
3	2	0	9,3
3	2	1	15
3	2	2	21
3	3	0	24
3	3	1	46
3	3	2	110
3	3	3	>110

APPENDIX 2: SHELF LIFE OF CULTURE MEDIA

Table 7: Example of physical properties and quality assessment of some media

MEDIA	PHYSICAL PROPERTIES		QUALITY ASSESSMENTS	
	pH	Shelf life	Productivity –test strains/ Target microorganism	Selectivity – test strains
Baird Parker Agar-BPA	6.9±0.1	Basal medium 3 Months at 20°C Complete medium 24hrs	*Stapylococcus aureus* 50081 *Stapylococcus aureus* 50080	*Escherichia coli* 50034
Baird Parker Liquid- BPL	6.6±0.2	Basal medium 1 Months at 4°C Complete medium 24hrs	*Stapylococcus aureus* 50080	*Escherichia coli* 50034
Bismith Sulphite Agar - BSA	7.6±0.2	Prepared medium 5 days at 4°C and 2days at 22°C	*Salmonella enteridits* 50073	*Escherichia coli* 50034
Brilliant Green Bile (BGB) Broth	7.4±0.2	Prepared medium 1 month at 4°C	*Escherichia coli* 50034	*Stapylococcus aureus* 50080
Enterobacteriaceae Enrichment Broth-EEB	7.2±0.2	Prepared medium 1 month at 4°C	*Escherichia coli* 50034	*Stapylococcus aureus* 50080
Frazer Broth – Modified -FBM	7.2±0.2	Basal medium 2 weeks at 4°C – use complete medium immediately	*Listeria monocytogenes* - serovar 1/2a (ATCC 35152/ NCTC 7973)	*Enterococcus feacalis* 50030
Hektoen Enteric Agar- HEA	7.2±0.2	Prepared medium 3 weeks at 4°C	*Salmonella enteridits* 50073	*Enterococcus feacalis* 50030
Lauryl tryptose broth-LST	6.8±0.2	Prepared medium 1 month at 4°C	*Escherichia coli* 50034	*Enterococcus feacalis* 50030
Oxford Agar and modified -MOX	7.2±0.2	Prepared medium 3 weeks at 4°C	*Listeria monocytogenes* - serovar 1/2a (ATCC 35152/ NCTC 7973)	Enterococcus feacalis 50030
Brilliant Green Agar -BGA	6.9±0.2	Prepared medium 1 month at 4°C	*Salmonella enteridits* 50073	*Escherichia coli* 50034
Rapport Vasiliadis broth -RVB	5.2±0.2	Prepared medium 6 month at 4°C in bottle	*Salmonella enteridits* 50073	*Enterobacter cloacae* (ATCC 23355)
Selenite Cystine Broth - SCB	7.2±0.1	Use immediate after preparation	*Salmonella enteridits* 50073 *Salmonnela typhimurium* (NCTC 12190)	*Escherichia coli* 50034 *Enterobacter cloacae* (ATCC 23355)
Thiosulphate Citrate Bile salt Sucrose Agar - TCBSA	8.6±0.2	Ready to use plates 2 weeks at 4°C	*Vibrio cholerae (non 01)* (NCTC11348 or *Vibrio parahaemolyticus (NCTC 11344)*	*Escherichia coli* 50034
University of Vermont broth Medium - UVM	7.2±0.2	Prepared medium 5 days at 4°C	*Listeria monocytogenes* - serovar 1/2a (ATCC 35152/ NCTC 7973)	*Enterococcus feacalis* 50030
Violet Red Bile Agars	7.4±0.2	Prepared medium 5 days at 4°C	*Escherichia coli* 50034	*Stapylococcus aureus* 50080 *Enterococcus feacalis* 50030
Xylose lysine Deoxycholate (XLD) agar	7.4±0.2	Prepared medium 5 days at 4°C	*Salmonella enteridits* 50073 Shigella flexneri 50079	*Enterococcus feacalis* 50030 *Stapylococcus aureus* 50080

Sorce: Corry J., (1995)

APPENDIX 3: VERIFICATION FORMS

FORM NO. 01:   **SAMPLE LOG FORM/BOOK**

Date	Customer/Client	Sample Description	Code nr	Comment	Log nr

FORM NO. 02:   **AUTOCLAVES RUN CYCLES VERIFICATIONS**
   Autoclave No.....................................Year..........

Date	Run nr	Run load details	T/t	Indicators	Comt	Sign
		BPW; PCA; LST; BGLB; EC; BPA; NA;	121°C 12mn 20mn	Autoclave tape Ver. Indicator		

FORM NO. 03:   **EQUIPMENT TEMPERATURE CHECK FORM**
(Including thermometers at ice/water boiling points and volume dispensers)

EQUIPMENT No:...Year..................
Parameter:...

Date	Jan	Feb	Marc	April	May	Jun	July	Aug	Sept	Oct	Nov	Dec
1												

31												

FORM NO. 04:   **CULTURE MEDIA PREPARATION FORM**

Date	Type	Lot nr.	weight /Vol	Final pH	Comment	Sign

Note: Enter lot number of each new opened media

FORM NO. 05:   **HUMIDITY RECORD SHEET**
                                 EQT NO…………

Date	Dry bulb	Wet bulb	Difference	Humidity	Remarks	Signature
1						

31						

FORM NO. 06:   **pH METER CALIBRATION CHECKS:**
                                 EQT NO………………………

Date	pH 4.0	pH 7.0	Comment	Sign	Date	pH 4.0	pH 7.0	Comment	Sign
1					1				
-----					------				
31					31				

FORM NO. 07: BALANCE CALIBRATION CHECKS

EQT NO………………………

Date	Level	Zero	+Ve weight	-Ve weight	Zero	Comment	Sign
1							

31							

FORM 08: DRY OVEN STERILIZATION CHECKS

Date	Glass-ware type	Time -start	Time - finish	Ster. tape	Sign
				Dry ster. tape	
				Dry ster. tape	

FORM NO. 09: LABORATORY ANALYSIS WORK SHEET

Sample description:..   Date:...

Analyst:..   Time:...

Head of laboratory:..

Sample	pH	Total Coliform (37°C)	Coliforms (37°C)	Feacal (44.5°C)	TPC 48h	Listeria monocytogenes (37°C)				S. aur (37°C)	V. cholerae (37°C)	Salmonella spp. (37°C)	
Log Nr	Te.	LST: 24/48h O 1 2 3	BGLB: 48h 0 1 2 3	EC : 24 0 1 2	22() 30() 37() °C	Frazer	M O X	C t	G c			TT	RV/ SEL
						2 4	4 8	4 8	5colony			BSA, XLD, HE	BSA, XL D, HE
												24 48	24 48
												24 48	24 48
Control 1		E.coli (-c)	E.coli (-c)	E.coli (-c)		Listeria	Li st					BSA: HE:	XLD:
Control 2		E.aerog.	E.aerog.	E.aerog.								TSI: B/S LIA: B/S	
Date													
Time													
Sign													

Legend: Ct = Catalase test; Gc = Gramm counting/ staining: (-C) = Negative control

Date/time/sign

Salmonella spp.	*Listeria*	*Vibrio spp.*
BPW (37°C):	UVM(30°C):	ABPW (37°C):
RV/SEL(42°C)/TT(37°C):	FRAZER (37°C):	
TSI/LIA (37°C):		

FORM 10: MANAGEMENT REVIEW MEETING FOLLOW UP ACTION PLAN

Date ………………………..

S/N	Agenda number	Action to be taken	Responsible person	Time frame	Verification

FORM 11:   AUDIT SCHEDULE

Area/Month	1	2	3	4	5	6	7	8	9	10	11	12	Date
Management requirements			⊗										
Technical requirements					⊗								

FORM 12:   INTERNAL AUDIT CHECKLIST

Date…………………………………

Clause of ISO 17025	Title of element and Descriptions	C	NC	*Detail of Non Conformance*	*Time frame for CA*

KEY:
C – Compliance   NC – Non compliance   CA – Corrective action

Confirmation of the findings

Auditor:…………………… Signature…………… Date:……………………

Head of Laboratory………… Signature ………… Date: …………………

FORM 13:   TRAINING PROGRAMME FOR NEW PERSONNEL

Name of trainee:…………………………………………………………
Area of work:……………………………………………………………
Name of trainer:…………………………………………………………

Type of Training	Training period	Contents	Name of trainer	Trainer's Recommendations	Trainer's Recommendations (after retraining)

Name of Trainee: …………………… Signature……………….. Date ………
Evaluation criteria: A trainee, whose test results of analysis do not deviate from the values of expected results, shall be deemed competent.
Verification by Head of laboratory:

Name………………. Signature…………………Date…………………………

APPENDIX 4: RAW RESULTS

LABORATORY TEST RESULTS

	Replicates	TPC-cfu/g	Sta. Aur.-cfu/g	Coliform-mpn/g	Enterob.
1	211	24000	7200	0,9	NIL
2	212	14000	8200	0,9	NIL
3	213	16000	4300	0,4	NIL
4	214	17000	9800	0,9	NIL
5	215	31000	15000	0,9	NIL
6	221	25000	9300	0,9	NIL
7	222	28000	5100	0,4	NIL
8	223	26000	11000	0,4	NIL
9	224	20000	7000	0,3	NIL
10	225	23000	5200	0,9	NIL
11	231	46000	16000	2,3	NIL
12	232	60000	14000	0,9	NIL
13	233	26000	13000	4,3	NIL
14	234	31000	15000	2,3	NIL
15	235	31000	11000	4,3	NIL
16	241	12000	3200	4,3	NIL
17	242	6800	2200	4,3	NIL
18	243	8300	1600	2,3	NIL
19	244	8000	2400	4,3	NIL
20	245	7900	2700	2,3	NIL
21	251	13000	5800	2,3	NIL
22	252	7800	2800	2,3	NIL
23	253	13000	7600	2,3	NIL
24	254	6800	2900	2,3	NIL
25	255	18000	15000	2,3	NIL

CONTROL SAMPLE

Replicates	TPC	Enterob.	S. aureus	Coliform	E. coli
1	1130000	540000	61000	75000	46000
2	1750000	123000	51000	7500	900
3	2540000	199000	59000	46000	4300
4	10000000	340000	48000	46000	9300
5	1270000	53000	25000	9300	4300
6	5700000	250000	67000	150000	4300