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CALIBRATION ACCORDING TO ISO/IEC 17025 IN THE OPERATION OF MICROBIOLOGICAL AND CHEMICAL LABORATORIES: AN EXERCISE IN CREATING CONTROL CHARTS FOR TPC AND TVB-N

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ABSTRACT

A study and practice of laboratory equipment calibration together with TPC and TVB-N methods of analysis according to ISO/IEC 17025 was conducted in order to gain and compile skills in calibration and internal quality checks of a laboratory using control charts in the two methods of analysis. The calibration facts and skills will be compiled into an equipment calibration manual while the two control charts will then give a rehearsal of internal quality checks of microbiological and chemical laboratories. All this is favourable to the Uganda Fisheries Laboratory as a step forward in gaining ISO7IEC 17025 accreditation to carry out analysis on fish and fish products for international export. In order to check performance of calibrated equipment and other quality parameters, which directly influence the microbiological results of analysis, a TPC control chart was obtained from the analysis of 10 frozen cod fillet samples for which the composite samples originated from the same consignment. The samples were prepared on the same day and then stored at -24ºC while inoculation was done along four consecutive days. Ten composite samples of shrimp muscle were prepared from 1000 g of a frozen consignment and then stored at -24°C and TVB-N analysis carried out during five consecutive days to obtain a control chart. This was aimed at checking the consistence of the analytical results in order to carry out internal quality checks focused on various factors that may directly or indirectly affect the certainty of the results. One of the controls was ensuring that all the equipment was calibrated before use and the other was keeping the samples at -24ºC to hold spoilage. From the control charts for TPC and TVB-N, the values from the sample fell within the acceptable region of the chart as expected.

Key words: ISO/IEC 17025 Laboratory, Calibration, TPC, TVB-N Analysis, Control Chart

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LIST OF ABREVIATIONS

CRMs- Certified Reference Materials
EA- European Co-operation for Accreditation:
EAL-European Co-operation for Accreditation of Laboratories
GUM [1] - Guide for Presentation of Uncertainty of Measurement
IEC - International Electro Technical Commission
IFL - Icelandic Fisheries Laboratories
ISO- International Standards Organisation
UKASTA-United Kingdom Agricultural Supply Trade Association
VIM1 - International Vocabulary of Basic and General Terms in Metrology

1 INTRODUCTION

Calibration of equipment is essential to be able to determine if instruments and equipment perform according to their respective specifications. It is a process of comparing objects, an unknown quantity with a known quantity, and if the difference between them is small, it is recorded, overlooked, or followed during subsequent measurements. If the difference is significant, action is taken to eliminate errors that may be caused by that difference through adjustment or exclusion of the difference, quantity, object, or equipment. That is to say if the previously unknown quantity is to be compared with unknown quantities to come. It involves correction of a value of a measure and by measuring a known value of that same quantity in a reference material (Biévre *et al.* 1997). This is one of the main quality requirements by the ISO/IEC17025 standard for calibrating and testing laboratories prior to accreditation to perform. Equipment calibration according to ISO/IEC 17025 is a quality control factor, which must be continuous, traceable and performed by personnel with the qualifications and skills to do so (ISO/IEC17025 clauses 5.2 and 5.5). This required training and the outcomes will be combined into a calibration manual to be used by Uganda Fisheries Laboratory and other microbiological and chemistry laboratories.

Reference materials and certified reference materials provide essential traceability in measurements and are used to demonstrate the accuracy of results, calibrate equipment, monitor laboratory performance, validate methods, and enable comparison of methods (ISO/IEC 17025 paragraph 5.6.3). Reference materials, can fulfil different functions, depending on the intended use. One of the functions located outside the traceability chain of a laboratory and hence not very relevant for establishing traceability is, evaluating the approximate size of the uncertainty of the measurement of an unknown sample by performing a similar measurement on a reference material (Biévre 1999). Since the uncertainty of each link in the traceability chain (measuring analytical instruments, reference material or other measurement standards) changes over time, the chain lifetime is limited. The lifetime in chemical analysis is dependent on the calibration intervals of the measuring equipment and the shelf life of the certified reference materials (CRMs) used for the calibration of the equipment.

Every laboratory should have a quality control system. Assembled into this system shall be a scheme for quality assurance to ensure that the laboratory performs tests correctly (Nordic Committee on Food Analysis 1994). To make the quality control effective it has to be harmonised, where by conformities have to be well outlined and implemented for accreditation. Apart from gaining accreditation, calibration and testing laboratories acquire various benefits, which include: improvement in all parameters considered in the standard, improved organisational learning through corrective and preventive activities, benefit from inter-laboratory proficiency tests and improved customer satisfaction (Halevy 2003).

Participation in appropriate proficiency studies or tests (an ISO/IEC 17025 requirement) enables laboratories to demonstrate the comparability of their measurements. If the materials used for the studies have traceable assigned values, then proficiency testing also provides information about measurement accuracy and confirms that appropriate traceability has been established. All values requiring calibration have to be calibrated and traceable in laboratory records or equipment labels in order to provide traceability in the laboratory quality system (Paul *et al.* 2003).

One of the inter-laboratory proficiency tests is a test carried out in a laboratory in order to create a control chart. This chart is then used to account for the personnel or equipment accuracy and other factors that may affect the quality of analytical results of the testing laboratory. The corrective and preventive activity considered in this study is equipment calibration, which is normally done by certified personnel.

ISO/IEC 17025 is a standard to emphasise and give guidelines or standards in the operation of an analytical or calibrating laboratory. Laboratories seek accreditation after fully implementing the requirements outlined in the ISO standard. The Uganda Fisheries Laboratory is seeking ISO/IEC 17025 accreditation, hence requires implementation of calibration and internal quality checks on analytical results as a prerequisite in seeking accreditation. If calibration and quality checks are not in place the results of analysis, however true they may be, will always be in doubt since there will be no traceable calibrated units and quality checks to verify the authenticity of analytical results. Total Plate Count (TPC) is a microbiological method, which is used to indicate the bacterial population of a sample while TVB-N is the total amount in mg of volatile (unstable) base nitrogen in 100 g of a sample and is a chemical method, which represents the quality of the fish products. These two methods of analysis were selected in this study because they are simple, basic (always used during monitoring the quality of fish) and generally cover most equipment that is used in food microbiology and chemical laboratories at the same time rational/sensible results are expected on all samples being tested using these methods.

The main objective of the study is to gain skills in calibration and internal quality checks using TPC and TVB-N control charts inline with ISO/IEC 17025 to be implemented in the Uganda Fisheries Laboratory as a step forward in acquiring ISO17025 accreditation and to have appropriate calibration and internal quality assessment of results during operation. This will be achieved through three main steps, which include:

Firstly, studying and carrying out equipment calibration in accordance with ISO/IEC 17025 in order to grasp and compile different calibration procedures into a collection of well-coordinated calibration steps, which may be used by the Uganda Fisheries Laboratory and other food microbiology and chemistry laboratories for which a calibration manual will be formed.

Secondly, studying and carrying out TPC and TVB-N analysis and create control charts for the two methods of analysis with the aim of assessing the deviation of test results for the composite samples. These deviations are caused by non-conformances in internal quality control for which poor personnel performance and testing equipment failure are assumed as the main causes among other ISO/IEC17025 requirements.

Finally, the implementation of quality checks on various laboratory equipment, working personnel and other factors outlined in the ISO/IEC 17025 standard in the Uganda Fisheries Laboratory by control charts and in the prospect follow the trend of international laboratory requirements for testing, calibration and external assessment (laboratory auditing). This will involve the use of certified reference samples and development of ring analysis (external laboratory checks) in Uganda as required by the current food laboratory diligence.

2 BACKGROUND INFORMATION

2.1 International standards and ISO standards

International standards are used for making manufacturing and supply of products and services more efficient, safer, and cleaner. They also make trade between countries easier and fairer. They can help governments with a technical base for health safety and environmental legislation. They can also help in transferring technology to developing countries.

ISO is a non-governmental organisation, network of the national standards institutes of 148 countries. The Central Secretariat is in Geneva, Switzerland and it coordinates the system. Although ISO is non-governmental, it occupies a special position between the public and private sectors because many of its member institutes are part of the governmental structure of their countries and it has members that have their roots uniquely in the private sector. Therefore, ISO is able to act as a bridging organisation of business.

ISO standards are of great importance for developing countries because there they can acquire technological expertise and raise their capability to export and compete in global markets (ISO 2003).

2.1.1 ISO 17025

The ISO 17025 standard specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, non-standard methods, and laboratory-developed methods. If laboratories comply with the requirements of the standard, they operate a quality system for testing and calibration that also meets the requirements of ISO 9001 and ISO 9002 (ISO 1999).

Laboratories were previously accredited against the European EN 45001 standard, in combination with the international ISO/IEC Guide 25. A new international standard was published in 2000 the ISO/IEC 17025. It contains all the requirements that testing and calibration laboratories have to meet if they wish to demonstrate that they operate a quality system, are technically competent, and are able to generate technically valid results.

Laboratories that previously met only the minimum requirements for accreditation have had some difficulty in meeting the requirements of the new standard. This is because ISO/IEC 17025 imposes more responsibility for quality work, improvement measures, and customer dialogue on the laboratories (Swedac magasinet 2003).

2.2 ISO/IEC 17025 requirements

The general requirements for accreditation are laid down in the International Standard; *General requirements for the competence of testing and calibration laboratories* (ISO/IEC 17025 1999), here after referred to as ISO 17025. The requirements include microbial and chemical testing procedures/ methods, their validity and the acquaintance of responsible personnel with these procedures, the equipment maintenance and calibration, materials, and culture media, environment that includes premises, environmental monitoring programmes, hygiene, and reflection of test conditions to test results. Other requirements include sampling procedures, sample handling, and identification, internal quality control and external quality assessment, documentation and labelling plus various requirements depending on laboratory magnitude and programme.

2.3 Equipment maintenance, calibration and performance verification according to ISO/IEC I7025

"As part of its quality system, a laboratory is required to operate a documented programme for the maintenance, calibration, and performance verification of its equipment. The documented programme enables follow up of equipment performance for as long as the equipment is in the laboratory working section" (ISO 17025, paragraph 5.5).

2.3.1 Maintenance and calibration

The laboratory must establish a programme for the calibration and performance verification of equipment, which has a direct influence on the test results. The frequency of such calibration and performance verification will be determined by documented experience and will be based on need, type, and previous performance of the equipment. Intervals between calibration and verification shall be shorter than the time the equipment has been found to take to drift outside acceptable limits. Examples of calibration intervals and typical performance checks for various laboratory instruments are given in Appendix D and Appendix E of the ISO 17025 standard. The equipment include:

a) Temperature measurement devices

Where temperature has a direct effect on the result of an analysis or is critical for the correct performance of equipment, temperature-measuring devices, e.g. liquid-in-glass thermometers, thermocouples and platinum resistance thermometers (PRTs) used in incubators and autoclaves, shall be of an appropriate quality to achieve the accuracy required. Calibration of devices shall be traceable to national or international standards for temperature. Where accuracy permits, devices that can be demonstrated to conform to an appropriate and nationally or internationally accepted manufacturing specification may be used (e.g. ISO 1770 for liquid-in-glass thermometers).

Such devices may be used for monitoring storage fridges and freezers. They are used for incubators and water baths where acceptable tolerance around the target temperature permits. Verification of the performance of such devices is necessary to limit their effect on the reality of results. Figure1 below shows digital thermometers giving different readings of the room temperature

Figure 1: Digital thermometers measuring room temperature (Professional equipment 2004).

b) Incubators, water baths, ovens

The stability of temperature, uniformity of temperature distribution and time required to achieve equilibrium conditions in incubators, water baths, ovens and temperature-controlled rooms shall be established initially and documented, in particular with respect to typical uses (for example position, space between, and height of stacks of petri dishes). The constancy of the characteristics recorded during initial validation of the equipment shall be checked and recorded after each significant repair or modification (EA 2002). Laboratories shall monitor the operating temperature of this type of equipment and retain records.

c) Autoclaves, including media preparators

These items affect the quality of results of analysis through direct or indirect contamination of samples or culture media provided the required steps are not followed when handling or using them.

The following outlines the generally expected approach to calibration and the establishment and monitoring of performance. However, it is recognised that quantitative testing of materials and items processed by autoclaving, able to comment suitably on variation within and between batches may provide equivalent assurance of quality.

(i) Autoclaves should be capable of meeting specified time and temperature tolerances. Pressure cookers fitted only with a pressure gauge are not acceptable. Sensors used for controlling or monitoring operating cycles require calibration and the performance of timers verified.

(ii) Initial validation should include performance studies (spatial temperature distribution surveys) for each operating cycle and each load configuration used in practice. This process must be repeated after significant repairs or modification (e.g. replacement of thermo-regulator probe or programmer, loading arrangements, operating cycle) or where indicated by the results of quality control checks on media.

Sufficient temperature sensors should be positioned within the load (e.g. in containers filled with liquid/medium) to enable location differences to be demonstrated. In the case of media preparators where uniform heating cannot be demonstrated by other means, the use of two sensors, one adjacent to the control probe and the other remote from it, would generally be considered appropriate. Validation and re-validation should consider the suitability of come-up and come-down times as well as time at sterilisation temperature.

(iii) Clear operating instructions should be provided based on the heating profiles determined for typical uses during validation/re-validation. Acceptance/rejection criteria should be established and records of autoclave operations, including temperature and time, maintained for every cycle.

(iv) Monitoring may be achieved by using a thermocouple and recorder to produce a chart or printout or direct observation and recording of maximum temperature achieved and time at that temperature.

In addition to directly monitoring the temperature of an autoclave, the effectiveness of its operation during each cycle may be checked by the use of chemical or biological indicators for sterilisation/decontamination purposes. Autoclave tape or indicator strips should be used only to show that a load has been processed, not to demonstrate completion of an acceptable cycle.

d) Weights and balances

Weights and balances shall be calibrated traceably at regular intervals (according to their intended use). Calibration procedures are well laid out for particular balances.

e) Volumetric equipment

(i) Volumetric equipment such as automatic dispensers, dispenser/diluters, mechanical hand pipettes and disposable pipettes may all be used in the EA - 4/10. Laboratories should carry out initial verification of volumetric equipment and then make regular checks to ensure that the equipment is performing within the required specification. Verification should not be necessary for glassware, which has been certified to a specific tolerance.

Equipment should be checked for the accuracy of the delivered volume against the set volume (for several different settings in the case of variable volume instruments) and the precision of the repeat deliveries should be measured.

(ii) For ‘single-use’ disposable volumetric equipment, laboratories should obtain supplies from companies with a recognised and relevant quality system. After initial validation of the suitability of the equipment, it is recommended that random checks on accuracy are carried out. If the supplier does not have a recognised quality system, laboratories should check each batch of equipment for suitability.

f) Other equipment

Conductivity meters, oxygen meters, pH meters, and other similar instruments should be verified regularly or before each use. The buffers used for verification purposes should be stored in appropriate conditions and should be marked with an expiry date. Where humidity is important to the outcome of the test, hygrometers should be calibrated, the calibration being traceable to national or international standards. Timers, including the autoclave timer, should be verified using a calibrated timer or national time signal. Where centrifuges are used in test procedures, an assessment should be made of the criticality of the centrifugal force. Where it is critical, the centrifuge will require calibration.

2.3.2 ISO/IEC 17025 performance verification

Quality assurance of results/quality control of performance:

a) Internal quality control

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 day-to-day results and their conformity with defined criteria. A programme of periodic checks is necessary to demonstrate that variability (i.e. between analysts and equipment or materials etc.) is under control. All tests included in the laboratory's scope of accreditation need to be covered. 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 construction of the programme and by the number of actual tests. It is recommended that, where possible, tests should incorporate controls to monitor performance. In special instances, a laboratory may be accredited for a test that it is rarely called on to do. It is recognised that in such cases an ongoing internal quality control programme may be inappropriate and that a scheme for demonstrating satisfactory performance, which is carried out in parallel with the testing, maybe more suitable. An example is the use of Total Plate Count Control Charts on a reference material if the laboratory is accredited for this test method and other methods, which apply similar test procedures (ISO 17025, paragraph 5.9).

b) External quality assessment (proficiency testing)

Laboratories should regularly participate in proficiency testing which are relevant to their scope of accreditation. Preference should be given to proficiency testing schemes, which use appropriate matrices. In specific instances, participation may be mandatory. Laboratories should use external quality assessment not only to assess laboratory bias but also to check the validity of the whole quality system (EA 2002). An example of an external proficiency test is the application of ring tests/analysis by accreditation bodies on a group of laboratories where reference material is used to construct charts to evaluate the performance of the laboratories. Proficiency or ring tests provide an independent external check against an established or reference value that the laboratory is achieving a consistent level of results, and that the results are in line with other facilities performing the same analyses (UKASTA 2003).

2.3.3 Items considered during performance verification

a) Reference materials

Reference materials and certified reference materials provide essential traceability in measurements and are used to demonstrate the accuracy of results, calibrate equipment, monitor laboratory performance, validate methods, and enable comparison of methods. If possible, reference materials should be used in appropriate matrices (conditions).

b) Reference cultures

Reference cultures are required for establishing acceptable performance of media (including test kits), for validating methods and for assessing/evaluating on-going performance. Traceability is necessary, for example, when establishing media performance for test kit and method validations. To demonstrate traceability, laboratories must use reference strains of microorganisms obtained directly from a recognised national or international collection, where these exist. Alternatively, commercial derivatives for which all relevant properties have been shown by the laboratory to be equivalent at the point of use may be used (ISO 17025, paragraph 5.6.3).

c) Uncertainty of measurement/ measurement uncertainty

The uncertainty of measurement is a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measureand (VIM1 and GUM [1]). The measurand is the material or object under measurement. The uncertainty of measurement is derived from the standard deviation of given results of analysis hence it is part of the result of a measurement. It is a measure for the accuracy of the result coming from sampling, instrument drifts and calibration, homogenisation and dilution effects, human factors, and environmental effects among others.

Measurement uncertainty is a requirement for testing and calibrating laboratories complying with ISO IEC 17025. For testing laboratories, the measurement uncertainty must be reflected in test results reports if it is required by the test method. The uncertainties are obtained from data stated in the standards. This includes:

- In house methods, which are through obtaining standard deviation from measurements using reference materials.
- Inter laboratory comparisons by obtaining the standard deviation from a great number of independent measurements under different conditions.
- Use of average control charts as quality tools for instruments, personnel, and methods. However, one should consider whether relevant reproducibility elements of uncertainty might be missing. This requires listing and controlling all the elements not covered during analysis to obtain data for the uncertainty of measurement. These elements influence the accuracy of measurement. A good example is avoiding change of media used.
- Estimation by experts on certain experiments reflects the uncertainty of an experiment, resulting from experience and knowledge.

Mathematical evaluation of measurement uncertainty:

The evaluation includes specification of measurand, mathematical expression of measurand and input qualities (by equation) and identification of all sources of uncertainty. Then input quantities are determined, standard uncertainties of all single components are quantified, covariance of input quantities are identified, the results of measurement is calculated from input quantities, and calculation of combined uncertainty, calculation of expanded uncertainty, the results of analysis together with the estimated uncertainty of measurement is given.

Different related formulae are used depending on the type of uncertainty evaluated. Below is type A used for calculation of standard uncertainties from a series of n measurements.

$$s(\bar{q}) = \frac{s(q_i)}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (q_i - \bar{q})^2}$$

where n = number of independent measurements
q_i = value of independent measurements for the determination of the input quantity x_j
u (x_j) = $s(\bar{q})$, the standard deviation is used directly as the standard uncertainty of the respective single component x_j, measured n times.

The international definition for uncertainty of measurement is given in ISO International vocabulary of basic and general terms in metrology, Appendix B 1993. The general approach to evaluating and expressing uncertainty in testing expected by European accreditation bodies is one based on the recommendations produced by the International Committee for Weights and Measures (CIPM), as described in the *Guide to the Expression of uncertainty in Measurement* (ISO/IEC CD 13240, "The Standard Multimedia/Hypermedia Scripting Language (SMSL)," ISO, Geneva, 1995. This provides requirements that a laboratory must have internal checks on measuring equipment and be able to record deviations from the reference scale required and then provide the values for uncertainty in measurements carried out in order to provide reliance for the analytical results obtained using these equipments.

d) Calibration intervals

The uncertainty of each link in the traceability chain (measuring analytical instrument, reference material or other measurement standard) changes over the course of time, hence the chain lifetime is limited. The lifetime in chemical analysis is dependent on the calibration intervals of the measuring equipment and the shelf life of the certified reference materials (CRMs) used for the calibration of the equipment. The ordinary least squares technique, used for treatment of the calibration data, is correct only when uncertainties in the certified values of the measurement standards or CRMs are negligible. If these uncertainties increase (for example, close to the end of the calibration interval or shelf life), they are able to influence significantly the calibration and measurement results. In such cases, regression analysis of the calibration data should take into account that not only the response values are subject to error, but also the certified values. As an end-point criterion of the traceability chain destruction, the requirement that the uncertainty of a measurement standard should be a source of less then one-third of the uncertainty in the measurement result is applicable (Ilya et al. 2000).

2.4 TPC and TVB-N methods of analysis

2.4.1 Total Plate Count

The Total Plate Count (TPC) is used as an indicator of bacterial populations in a sample. It is also called aerobic colony count, standard plate count, mesophilic count or aerobic plate count. The test is based on an assumption that each cell will form a visible colony when mixed with agar containing the appropriate nutrients. Pour plate method is also a TPC method. Although this technique can have limitations in enumerating microorganisms through colony count, many of the errors can be eliminated if the analyst follows directions carefully and exercises extreme care in taking all measurements (Morton 2001).

2.4.2 Total Volatile Base Nitrogen (TVB-N)

The most commonly used biochemical methods for assessing spoilage are TVB-N and TMA. The amount of these compounds allowed in fish muscle is regulated according to Directive (95/149/EEC) and Directive (91/493/EEC), respectively. These biochemical indexes are determined in trading practice and sanitary inspection when the sensory analysis results are open to doubt, because there is a high correlation between these parameters in some species of fish (Ruiz-Capliias 1997 and Simeonidou *et al.* 1998).

2.5 Control charts and their applications

Control charts are a powerful tool for the statistical control of analytical methods. They are capable of detecting irregularity when the analytical process has gone out of control giving abnormal results. This can be due reduced equipment performance or other factors mentioned in the ISO/IEC 17025 standard. The construction consists of several steps. Based on the position of the points in the control chart, the state of the analytical system under examination can then be assessed (O.Mestek *et al.* 1997) not on list.

The foremost /original charts were the Shewhart control charts. The first chart was developed by W.A. Shewhart in the 1920s. The simplest control chart consists of plotting the measured data on the vertical axis versus the order (or time) in which those data are obtained on the horizontal axis (Rius *et al.* 1998). These charts have found wide application in analytical laboratories. Their construction consists of recording the central line (CL) corresponding to the expected value of the parameter under study and two limit pairs about this central line, viz. the upper and lower warning limits (WL) and upper and lower action limits (AL). This approach enables the control charts for the parameter of location (accuracy control chart) and the parameter of dispersion (precision control chart) to be plotted (O.Mestek *et al.* 1997)

2.5.1 Construction of control charts

The laboratory management should ensure that the results from the reference samples are put in the control chart directly after analysis to give management a clear and early indication of whether the results keep within the limits or not.

1. Analysis of 10 to 20 reference samples is done (more samples give better results). All personnel who work at the laboratory should, if possible, participate in the analyses. This will eliminate the risk of under representation of personnel.
2. A chart is drawn for each analysis and a mixture of reference samples is drawn. The date of analysis is put on the x-axis and the result, log no. bacteria/ml (microbial) or quantitative vale for chemical analysis on the y-axis.
3. The mean value and standard deviation of the results of each analysis are calculated and the mean value is transferred into the diagram.
4. The limits are calculated, the so-called warning and control limits, 2x the deviation, and 3x the standard deviation. As these limits are transferred into the control chart. The chart is now ready to be used.
5. The results from individual analyses, workers or laboratories are filled continuously into the control chart.

(Nordic Committee on Food Analysis/NMKL 1997).

2.5.2 Activities after construction of the control chart

The control chart is a diagram, where the results of analyses from known control samples are continuously filled in, has upper and lower control limits that the results of analysis should be within. Therefore, it is advisable to record and store the results from the reference samples. This calls for documentation of the outcomes and assigning corrective actions after interpretation of results and identification of non-conformances. Any source of error that may occur must be identified and dealt with promptly.

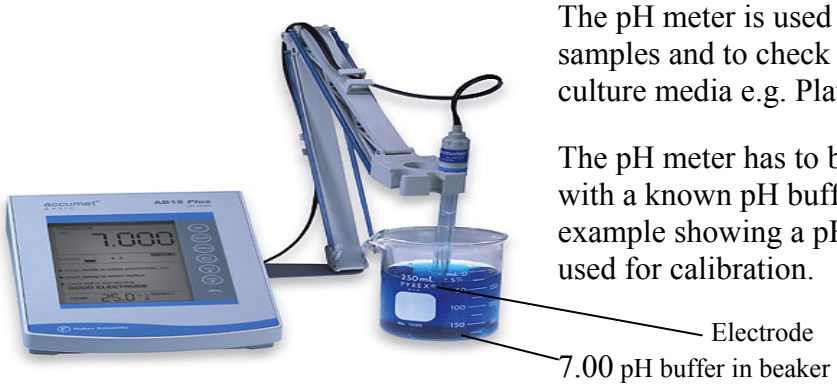
Tolerance intervals in the enclosed certificate of the reference samples show the limits, which the specific values should keep within to be considered true. Estimating the intervals, the variations within as well as between laboratories have been taken into consideration in this certificate. Some laboratories end up in the lower part of the interval while others will end up in the upper part of the interval, which is completely normal. The spread of results origin from one specific laboratory should be considered less than the interval given in the certificate. These broad intervals should be used as limits of error for a specific laboratory. Each laboratory should construct a control chart based on their own limits of error estimated from their own results (Nordic Committee on Food Analysis/NMKL 1997).

Uncertainty of measurement resulting from equipment, test methods, personnel and other factors can be evaluated from control charts. However one should consider whether relevant reproducibility elements of uncertainty are missing (European Federation of National Associations of Measurement 2002).

3 MATERIALS AND METHODS

3.1 Calibration

3.1.1 pH meter calibration



The pH meter is used to measure pH in samples and to check the pH of prepared culture media e.g. Plate Count Agar.

The pH meter has to be calibrated daily with a known pH buffer. Figure 2 is an example showing a pH buffer of pH 7.00 used for calibration.

Figure 2: Bench top pH /mV meter on online sale at Cole-Parmer an international company for laboratory equipment and technical information.

Description of the daily calibration of the pH meter:

Precautions:

1. The electrode is kept in demineralised water. The water is changed daily.
2. It is ensured that the lower part of the electrode contains enough potassium chloride (KCl) crystals.
3. The electrode is cleaned using a mild soap solution and then rinsed with water to remove dirt.

Materials:

Buffer solutions pH 4.01 and 7.00
Demineralised water
Mild soap solution
Potassium Chloride solution
Calibration logbook

Daily electrode performance check (a magnetic stirrer was used during measurements):

1. The electrode was rinsed with demineralised water and then with fresh pH 7.00 buffer.
2. The electrode was immersed in a fresh pH 7.00 buffer. Adjustment was done on the buffer knob. The appropriate box was ticked in the calibration logbook.
3. The mV value was recorded. It was considered to be in the region -30-+30 mV.
4. The electrode was rinsed with demineralised water and then with fresh pH 4.01 buffer.
5. The electrode was immersed in fresh pH 4.01 buffer. Adjustment was done as necessary (using the set temperature button). The appropriate box was then ticked in the logbook.

6. The mV value was recorded: It was approximately 160 mV higher than the value obtained at pH 7.00, as it should be.
7. The electrode was checked to see whether it was performing properly or not (for replacement or continued usage).

3.1.2 Analytical balance calibration

There are several balances in both the chemical and microbiology laboratories. These balances are checked weekly with at least two working weights (M1, F1, or F2). The laboratories have calibrated weights that are traceable to international standards. These weights, which are called working weights, are solely used for performance and routine checks of balances.

They are recalibrated every five years.

Weekly check

This is an example of a weekly check of Metter PG- 2002 balance located in the microbiological laboratory of IFL.

Apparatus and materials:

International calibration weights of three classes: M1, F1, and F2 contained in special calibration cases with, special gloves, forceps as shown in Figure 3 taken at the Icelandic Fisheries Laboratories.

Figure 3**: Calibration weights M1 & F2.**

Precautions:

1. The balance is smoothly separated and the loose parts cleaned with special cloth from the calibration case and reassembled to prevent interference of movement and elimination of alien weights as shown in Figure 4 taken during calibration for balance PG 2002 at the Icelandic Fisheries Laboratories.

Figure 4**: Cleaning the balance.**

2. The level of the balance is checked to ensure it is in the horizontal plane on the table, then that it is permanent in place.

3. It is emphasised not to touch weights without gloves and not to touch any thing with gloves except the weights.

4. Dropping weights is not acceptable.

5. The weights are placed gently on the balance

Procedure:

The following weights were put on the balance; the resultant reading was checked and recorded in a calibration logbook.

Mass of weight: 1 g (F2). Acceptable limits: 1.00-1.00 g (Figure 5).

Mass of weight: 100.00 g (FI). Acceptable limits: 100.00-100.00 g (Figure 6).

Mass of weight: 2000.00 g (M1). Acceptable limits: 1999.90-2000.10 g (Figure 7).

Figure 5: **1.00 g load.**

Figure 6**: 100.00 g load.**

Figure 7: **2000.00 g load.**

Procedure for one weighing scale in the microbiology laboratory (Metter PG 2002):

i) Measurement (without taring):

2000 g M1 (2000 g weight) was put on the balance.
The reading was taken.
100 g F1 were put on the balance and the reading was recorded.
1 g F2 (1 g weight) was put on the balance and the reading was recorded.

ii) Measurement (with taring):

100 g weight was put on the balance and then tarring was done.
2000 g M1 (2000 g weight) was put on the balance, then the reading was recorded.

100 g F1 (100 g) was put on the balance and the reading was taken.
1 g F2 (1 g weight) was put on the balance then the reading was taken.

Once a year the balances are thoroughly inspected.

The performance inspection once a year includes the following:

(a) The hysteresis and linearity of the balance is checked through the whole scale by different loading starting around the maximum capacity and going down to the approximate minimum loading capacity and back up again to the maximum capacity. At least five points (100, 75, 50, 25, 0% of the maximum capacity) are measured,

evenly divided throughout the scale. Each point is measured twice, i.e. once when going down the scale and once more when going up the scale. The average of the two measurements is calculated and the deviation is calculated. It is not allowed to exceed the maximum permissible deviation of the weight.

(b) Repeatability is where the mass of calibrated working standards, which is a weight, is measured at least three times in a row. The balance is allowed to reach equilibrium between each individual measurement. This is done with at least five points (100, 75, 50, 25, 0% of the maximum capacity). From these results the average, standard deviation, measuring uncertainty and the deviation of each weight is calculated and compared to the same in the calibration report of the weight. Maximum permissible deviation is defined and is not to be exceeded.

(c) Eccentric loading is where one weight near to 1/3 of the loading capacity of the balance is used. The measurement starts at point 1, i.e. the middle of the weighing pan. Thereupon the weight is put on four imaginary corners of the pan. The balance is in each instance allowed to reach zero between different measurements. The results at the four imaginary corners are compared to the results when the weight is put into the middle of the pan. The deviation, as compared to the measuring in the middle of the pan, is calculated and it is not allowed to exceed the maximum permissible deviation of the weight.

(d) Tarring and sensitivity: Tarring is checked with about the maximum and the half-maximum loading capacity of the balance whereupon the mass of several weights is checked having in mind how the balance is used as a rule in the routine work of the laboratory. The sensitivity is checked by tarring the balance with approximately the maximum loading capacity and the mass of small weights near to the minimum loading capacity is checked, i.e. if the weight can weigh down to 10 mg, we weigh down to between 20 and 50 mg. The deviation is then calculated.

Results of the inspection once a year are represented in a calibration report. The calibration report has to have its own number, to be dated and signed. Results of all measurements during the calibration are reported. Further evaluation as to calculations of the average, standard deviation, uncertainty of measurement, correction, or deviation from the supposed real value as reported in the calibration certificate.

Example:

This is an example of a performance inspection of Metter PG- 2002 balance located in the microbiological laboratory of IFL.
Write down room temperature with 0.5°C accuracy.
Check if the balance has been turned on for at least 30 minutes.
Check if the balance is standing right and clean it if necessary.
2 kg M1 (2000 g weight) put on and the reading written down in logbook.

i) Inner calibration

Hold -CAL-button down until -CAL int- is seen, then release button.
Finally comes Cal done and then 0.00 g.
2000 g is put on and the reading written down in the logbook.
ii) Hysterisis and linearity

These weights are put on and the reading written down:

2100 g M1 (2000 g weight) + F1 (100 g weight) 100%-maximum
1575 g M1 (1000 g weight) + F1 (500 g weight) +F2 (50 g + 20 g + 5 g weight) 75%
1050 g M1 (1000 g weight) + F2 (50 g weight) 50%
525 g F1 (500 g weight) + F2 (20 g + 50 g weight) 25%
0,01 g F2 (10 mg weight) 0% minimum

Repeat and go up the scale from 0.01 g - 2100 g.

iii) Repeatability

Same weights were measured 3 times in a row
2100 g M1 (2000 g weight) + F1 (100 g weight) x3
1575 g M1 (1000 g weight) + F1 (500 g weight) + F2 (50 g + 20 g +5 g weight) x3
1050 g M1 (1000 g weight) + F2 (50 g weight) x3
525 g F1 (500 g weight)+ F2(20 g + 50 g weight) x3
0.01 g F2 (10 mg weight) x3

iv) Exentric loading (done on analytical balance PG 2002 of IFL)

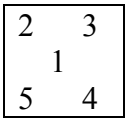


Figure 8**: Top of balance showing order of placement of the weights.**

700 g weight (1/3 of loading capacity) F1 (500 g + 200 g weight). Loading was started in the middle of the pan (1) reading written down. The balance was allowed to reach zero between different measurements. There upon the weight was put on four imaginary corners of the pan. (2-5).the reading was written down and the balance allowed reaching zero between different measurements.

v) Tarring and sensitivity:

Tarring was done with 2000 g M1 (2000 g weight).
Weights 50 g,20 g,5 g,1 g,0.1 g (F2 weights) were put on the balance and the balance allowed to reach zero between different measurements.

Tarring was also done with 700 g F1 (500 + 200 g weights)
50 g, 20 g, 5 g, 1 g, 0.1 g weights were put and the balance allowed to reach zero between different measurements.

In the end, the room temperature was noted.

All results of the weighing were recorded in a quality assurance logbook.

3.1.3 Thermometer calibration

i) Reference thermometers:

The laboratories have calibrated reference thermometers, which are calibrated by an internationally accredited laboratory and traceable to international standards. These reference thermometers are recalibrated every five years (mercury glass thermometers) and every two years (digital thermometers).

The calibrated reference thermometers are solely used for calibration of working thermometers, which are used to check the temperature of incubators, water baths, and autoclave.

ii) Calibration of working thermometers:

Apparatus and materials:

Calibrated reference thermometer (Figure 9 below).
Calibration system (Water or oil bath Figure 11 below)
Beaker, 250 ml.
Dewar flask (Figure 10 below).
Ice
Water

Figure 9**:** Calibrated i**nternational reference thermometer.**

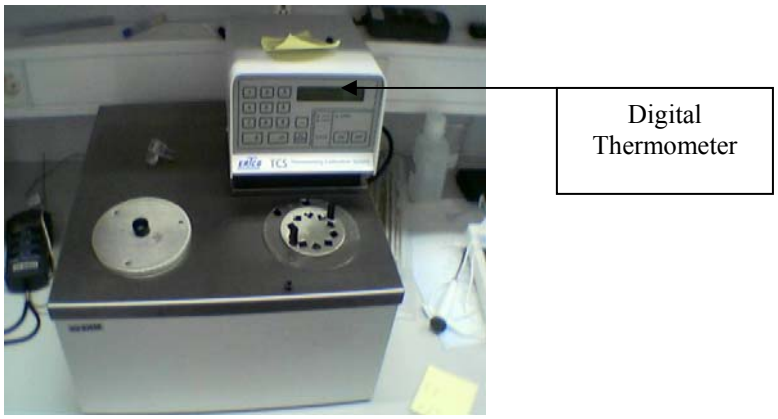


Figure 10: Calibration system with oil/ water bath for Icelandic Fisheries Laboratories.

Figure 11**: Set of two Dewar flasks (one of them is used at a time specifically for sub-ambient temperature calibration shown in part (iv) below) (Cole-Parmer 2004).**

Precaution:

The international reference liquid in the glass thermometer should be handled with concern since it is fragile and costly. The glass thermometer should be calibrated every five years while the digital every two years.

If part of the Dewer flask is glass, (Figure 11) it is also fragile.

Procedures for different thermometer calibrations:

i) Ambient temperature calibration:

A 250 ml beaker is filled with tap water or oil and allowed to equilibrate to room temperature for one hour.
The bath is turned on, adjusted to the room temperature, and allowed to equilibrate for one hour.
Both the reference thermometer and the working thermometers are inserted into the beaker and the readings are allowed to stabilise.
The temperature readings of both thermometers are recorded in a work sheet.

ii) Elevated temperature:

Water can be used if the bath medium is below 95°C. Over that, oil should be used as the medium. Oil can also be used below 95°C.
The hot water or oil bath is turned on, adjusted to the right temperature and allowed to equilibrate for one hour.
Both the reference thermometer and the working thermometer are inserted into the bath and the readings allowed to stabilise.
The temperature readings of both thermometers are recorded.

iii) Sub-ambient temperature:

An ice bath is prepared in a suitable Dewer flask, like one of the two shown in Figure 11 above, by adding shaved ice and distilled water. It is allowed to reach a constant temperature, any excess water is removed and more ice added. A ready ice bath should have no floating ice and excess water on the surface. Both the reference thermometer and the working thermometer are inserted into the flask and the readings allowed to stabilise. The temperature readings of both thermometers are recorded.

iv) Calibration of the autoclave thermometer:

Maximum thermometers are used. The reference thermometer (Figure 12 below) and the working thermometer are put into a container with cotton in the bottom to avoid breaking as shown in Figure 13. The meters stand at a 45° angle. Then the container with the meters is put in the autoclave and an autoclave run at 121°C for 50 minutes is done (Figure 13). The meters are taken out of the autoclave after the run and let to stand at room temperature for three minutes before the reading is taken as shown in Figure 14. After calibration the working thermometers are marked with the correction necessary and the time of calibration and recalibration.

Figure 12**: Maximum reference thermometer.**

Figure 13: Meters in autoclave before reading is taken.

Figure 14: Meters stand out for three minutes.

v) Daily check of water bath, autoclave, and incubator:

In the Total Plate Count method, the water bath is used to keep the melted Plate Count Agar at 44-45°C. The incubator is used to incubate the bacteria at 30°C and the autoclave is used to sterilise the agar and the dilution water.

Water bath:

A calibrated working thermometer is situated in the water bath. The thermometer is read at the start of each working day, before the bath is opened for the first time and the reading was registered on a form on the lid of the bath Figure 15.

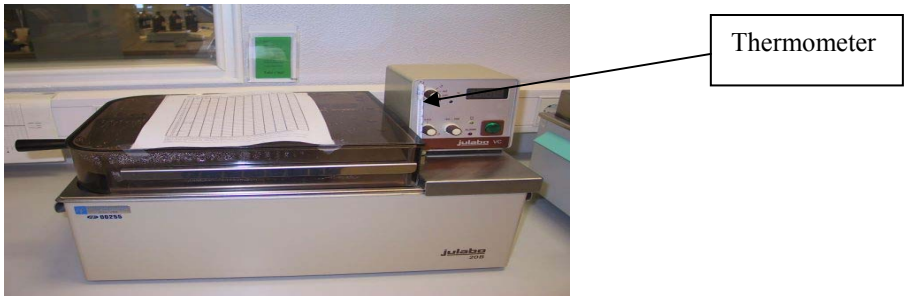


Figure 15**: Water bath (picture taken at the Icelandic Fisheries Laboratory).**

At all times, it was checked that the water bath was adequately filled with water; the level was monitored in order for it not to go below the upper content level of the

incubator tubes. Demineralised water, heated to 44.5˚C, was added to the water bath whenever it was necessary. It was noted in the quality manual that the water bath must be cleaned at least once per year.

Autoclave:

Each time a sterilisation is carried out in the autoclave, an autoclave tape is glued to bottles, glass stands and other commodities placed into it. The date and run number must be registered on the tape. If the sterilisation is satisfactory, dark stripes appear on the tape. Once per day a Browne TST strip is used; a colour change from yellow to violet indicates satisfactory sterilisation. Twice per month, the maximum thermometer is placed in the autoclave chamber during a sterilisation. Once the sterilisation is completed it should indicate 121±2°C. Four times per year a BBL® spore ampoule (Becton Dickinson-agent) which contains live *Bacillus stearothermophilus* spores, is placed in the autoclave during sterilisation. After sterilisation, the ampoule is incubated at 56-60°C for up to seven days along with an unheated ampoule (as a control). If growth occurs, the solution becomes cloudy and turns from violet to yellow. If the heated ampoule becomes yellow the sterilisation was unsatisfactory. The autoclave is cleaned when necessary. A service agent maintains the autoclave four times per year and repairs it when necessary.

Incubator 30˚C:

The thermometer inside the incubator is read only once per day. During the study, the readings were recorded. The meters were read in the morning when the incubator was run for the first time before being used. Precaution was taken that the incubator must have been closed for at least a half an hour before the reading was taken as required. The working thermometer in the incubator was immersed in paraffin oil for appropriate heat transfer.

The thermometer has to be used according to specification since its function depends on the difference between the coefficients of expansion between the glass and the liquid used. If the specifications are not followed then the liquid in glass thermometers do not measure correctly.

Categories of thermometers by use:

i) Total immersion:

Used when the measured media and container are transparent where the scale can be read through the container and the media where the thermometer is immersed measured. A total immersion thermometer is designed to indicate temperatures correctly, when the bulb and the entire liquid column are immersed in the substance being measured. A minimal length of the thermometer must be visible.

ii) Partial Immersion:

Used when measuring opaque containers or media where the scale cannot be read through the container or media. A partial immersion thermometer has a line around it indicating maximum immersion depth. For maximum accuracy, select the proper thermometer. A total immersion style offers the best accuracy when used properly. A partial immersion style is appropriate for use when immersion depth is limited. When used correctly, either type will yield reliable results. A total immersion thermometer can be used accurately at partial immersion if the following correction is applied:

T-t) 0.000089°F x N
(T-t) 0.00016°C x N
T = temperature of the bath
t = average temperature of stem
N = number of degrees emergent

Figure 16**: Partial and total immersion liquid in glass thermometers.**

Calibration using reference thermometers:

iii) Reference thermometers:

Calibrated by an internationally accredited laboratory, every five years for liquid in glass thermometers and two years for digital thermometers.

iv) Working thermometers:

These are used for general temperature measurements for water baths, refrigerators, autoclave, incubators, oven and measurements within or outside the laboratory. They are calibrated using the reference thermometer and calibration equipment described above.

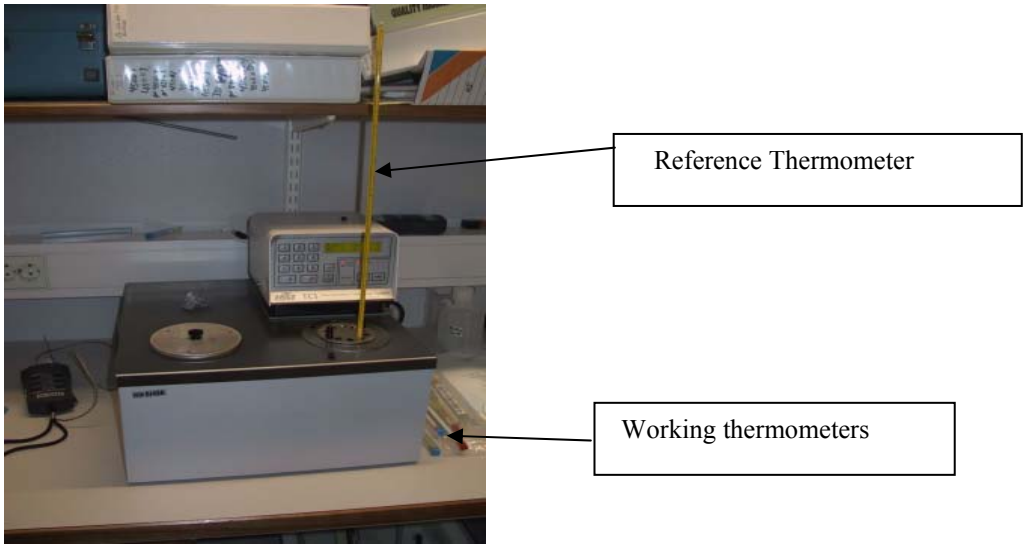


Figure 17**: Oil bath**

3.2 Total Plate Count

3.2.1 TPC apparatus and materials

a) Apparatus

pH meter
Autoclave
Mincing machine
Plastic bags
300 ml beakers
Stomacher
Balance
Colony counter
Dilution test tubes
Petri dishes
10 ml & 1 ml Pipettes
Pipette aids (pump)
Refrigerator
Reference thermometers for autoclave, refrigerator, water bath
Work area (clean, level table)
Water bath
Incubator

b) Materials

Cod fish fillet samples
TPC Agar (Preparation 23.5 g PCA, 5 g salt and 1 l of water)

3.2.2 Total Plate Count (TPC) method

For the first week, 10 fillet samples were prepared from the same consignment and tested through incubation at TPC, 10 analysis results for TPC after 48 hours were gathered to obtain a control chart.

Preparation of Agar:

23.5 g Plate Count Agar + 5 g salt + 1 l of distilled water. The mixture is heated to boil and autoclaved at 121ºC for 15 minutes.

Sample preparation:

Sea frozen cod fillets were minced and 10 25 g samples prepared in stomacher bags and stored in a laboratory freezer at -24˚C .Two samples were taken out of the freezer and left to stand for 15 minutes and then 225 g of Butterfield's buffer were added to make a 1/10 sample dilution and stomached for 1 minute. At this step, two duplicate dilutions were made. Three more duplicate dilution steps were done with test tubes using the dilution buffer. Eight Petri dishes were used to represent the four dilution steps in pairs. Inculcation was done for the four dilutions after which melted Plate Count Agar (45˚C) with 0.5% Nacl was poured on the plates and the contents mixed. All the plates were incubated inverted at 30±0.5˚C for 48 hours. Counting was done as described in the next section.

Counting colonies:

Prior to counting, the plates were put in the right dilution order, to check whether there was a normal tenfold difference between dilutions. Counting was done with a double magnification in a Quebec Colony Counter and a hand tally was used to aid the counting. Plates from the dilution showing colony numbers 25-250 were chosen for counting. For the two plates chosen, colonies from both sides were counted; the mean of both was found and multiplied with the dilution factor to get the final microbial content in the original sample (American Public Health Association 1992, Food and Drug Administration 1995) . In all steps in this method of analysis, equipment and personnel have a connection to the quality of the final values of analysis. This can be through temperature, pH, weight, and skills in both the calibration of equipment used and the method of analysis among other factors.

3.3 TVB-N materials and methods

3.3.1 TVB-N apparatus and materials

a) Apparatus

Waring blender
Whatman n° 3 paper
Funnel
200 ml bottles
25 ml & 10 ml pipettes
Distillation flask
Kjeltec type distiller (Vapodest Gerhardt)
0.01 graduated micro burette

b) Materials

kg Shrimp muscle (Figure 18)
7.5% aqueous trichloro acetic acid
10% NOAH
4% Boric acid and methyl red and bromocresol green indicator
Aqueous 0.25N sulphuric acid solution

Figure 18**: Frozen shrimp muscle batch and blender.**

Figure 19**: Frozen shrimp samples F3 & F4**

3.3.2 TVB-N analysis (chemical analysis method)

100 ml of 7.5% aqueous trichloro acetic acid was added to 50 g of shrimp muscle (Figure 19) and homogenised in a Waring blender. The mixture was filtered through Whatman n° 3 paper after 10 minutes. 25 ml of filtrate were transferred into a distillation flask and 6 ml of 10% NOAH were added. Steam was produced by using Kjeltec type distiller (Vapodest Gerhardt) shown in Figure 19. A beaker containing 10 ml of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the condenser for the titration of ammonia. Distillation was started and

steam distillation continued for 4 minutes. The boric acid solution turned green when alkalinised by the distilled TVB-N (see conical flask in Figure 20), which was titrated with aqueous 0.3003N sulphuric acid solution using a 0.01, graduated micro burette. Complete neutralisation was obtained when the colour turned pink on the addition of a further drop of sulphuric acid (Malle *et al*. 1998). As shown in Figure 20 below, all measurements in quantities of materials and time have an effect on the final value of TVB-N analysis. Equipment or personnel carrying out the test always cause the effect.

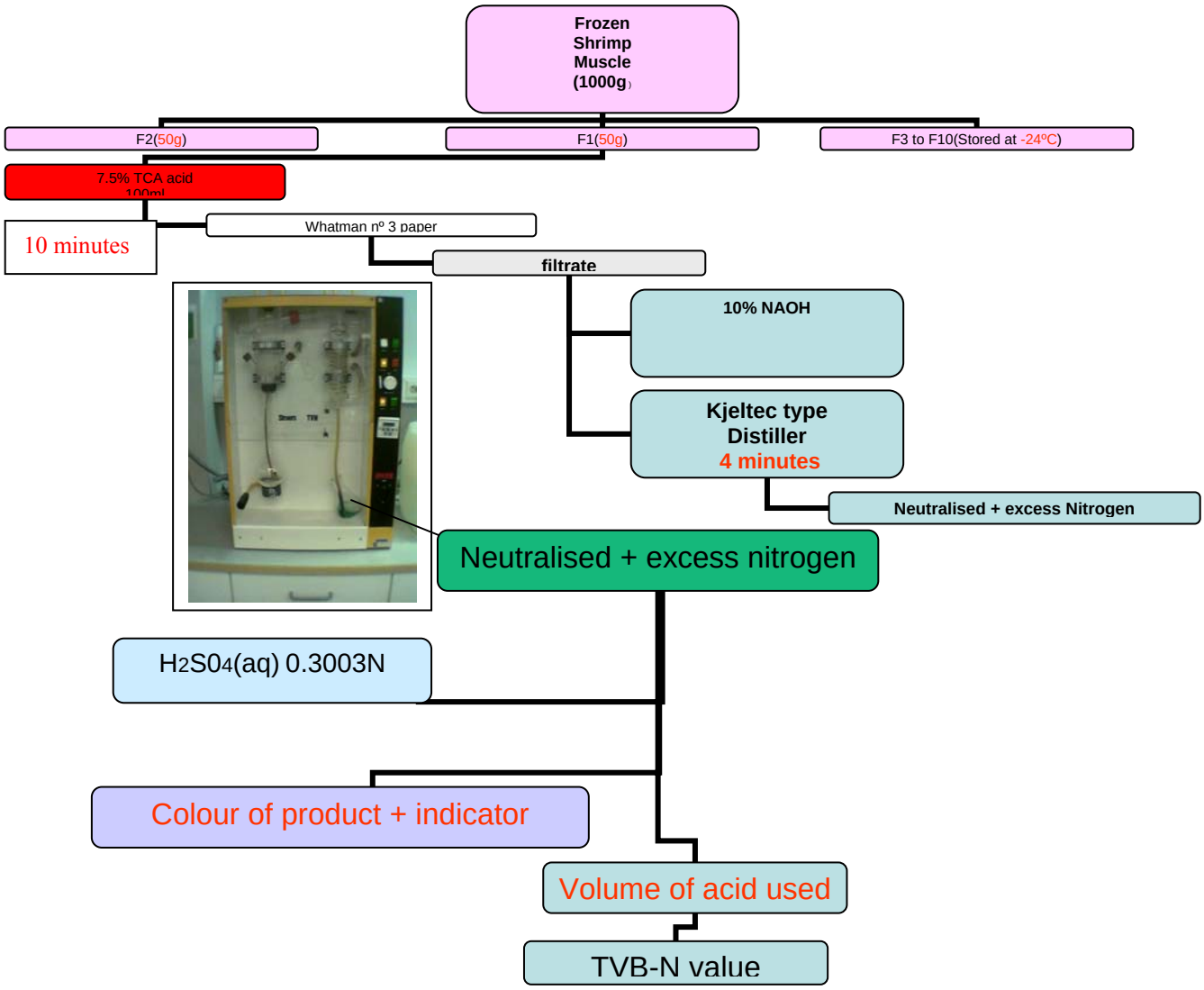


Figure 20**:** Flow diagrams for TVB-N analysis & **Kjeltec type distiller in set.**

4 RESULTS AND DISCUSSIONS

4.1 Results for TPC analysis

Table 1**:** Results for TPC from 10 composite samples.

Sample code	Day	Date	Log bacteria (x)	(x-mv)	$(x-mv)^2$
F1	1	3/12/2003	4.13	0.35	0.12
F2	1	3/12/2003	4.04	0.26	0.07
F3	2	8/12/2003	3.93	0.15	0.02
F4	2	8/12/2003	3.50	-0.29	0.08
F5	2	8/12/2003	3.83	0.05	0.00
F6	2	8/12/2003	3.75	-0.04	0.00
F7	3	9/12/2003	3.65	-0.14	0.02
F8	3	9/12/2003	3.35	-0.43	0.19
F9	4	10/12/2003	3.62	-0.16	0.03
F10	4	10/12/2003	4.04	0.25	0.06
n=10			**$\sum x$=37.84**		**$\sum(x-mv)^2$=0.58**

For the method of construction of control charts see section 2.6.1.

x = Log values for samples F1, F2, F4, F5, F6, F7, F8, F9 and F10
mv = mean value for all TPC analyses = **$\sum x/n$ =3.78**
s = standard deviation = **$\sqrt{\sum(x-mv)2/n-1}$ = 0.26**
Upper control/action limit (UCL/UAL) = mv+3s = 4.55
Upper warning limit (UWL) = mv+2s = 4.30
Lower control/action limit (LCL/LAL) = mv-3s = 3.02
Lower warning limit (LWL) = mv-2s = 3.30

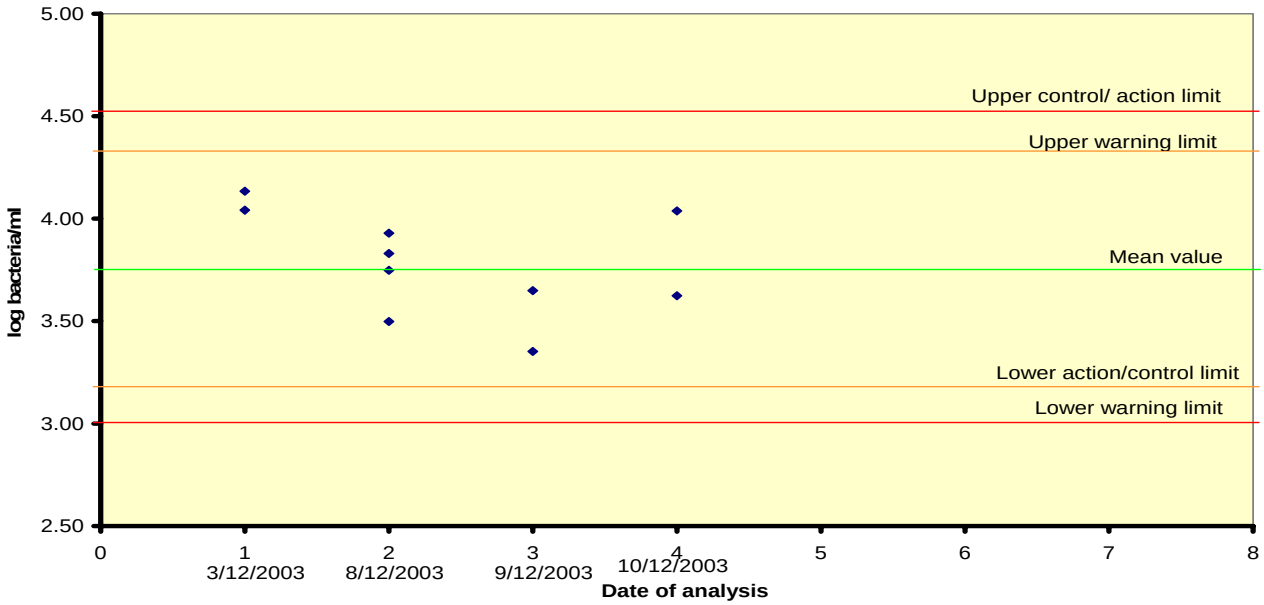


Figure 21**:** TPC control chart.

4.2 Results for TVB-N analysis and discussions

Table 2: Results for TVB-N from 10 composite samples.

Sample code	Day	Date	TVB-N (mg/100 g)(x)	(x-mv)	$(x-mv)^2$
F1	1	5/1/2004	11.94	0.20	0.04
F2	1	5/1/2004	12.41	0.68	0.46
F3	2	6/1/2004	11.96	0.23	0.05
F4	2	6/1/2004	13.29	1.55	2.41
F5	3	7/1/2004	11.86	0.12	0.02
F6	3	7/1/2004	11.48	-0.26	0.07
F7	4	8/1/2004	11.48	-0.26	0.07
F8	4	8/1/2004	11.35	-0.38	0.15
F9	5	9/1/2004	10.72	-1.01	1.02
F10	5	9/1/2004	10.85	-0.89	0.78
n=10			**$\sum x=117.32$**		**$\sum(x-mv)^2=5.06$**

For the method of construction of control charts see section 2.6.1.

n=10, $\sum x = 117.32$ and $\sum(x-mv)^2 = 5.07$
x = Log values for samples F1, F2, F4, F5, F6, F7, F8, F9 and F10
mv = mean value for all TVB-N analyses = **$\sum x/n = 11.73$**
s = standard deviation = **$\sqrt{\sum(x-mv)2/n-1} = 0.75$**
Upper control/action limit (UCL/UAL) = mv+3s = 13.98
Upper warning limit (UWL) = mv+2s =13.23
Lower control/action limit (LCL/ LAL) = mv-3s = 9.48
Lower warning limit (LWL) = mv-2s = 10.23

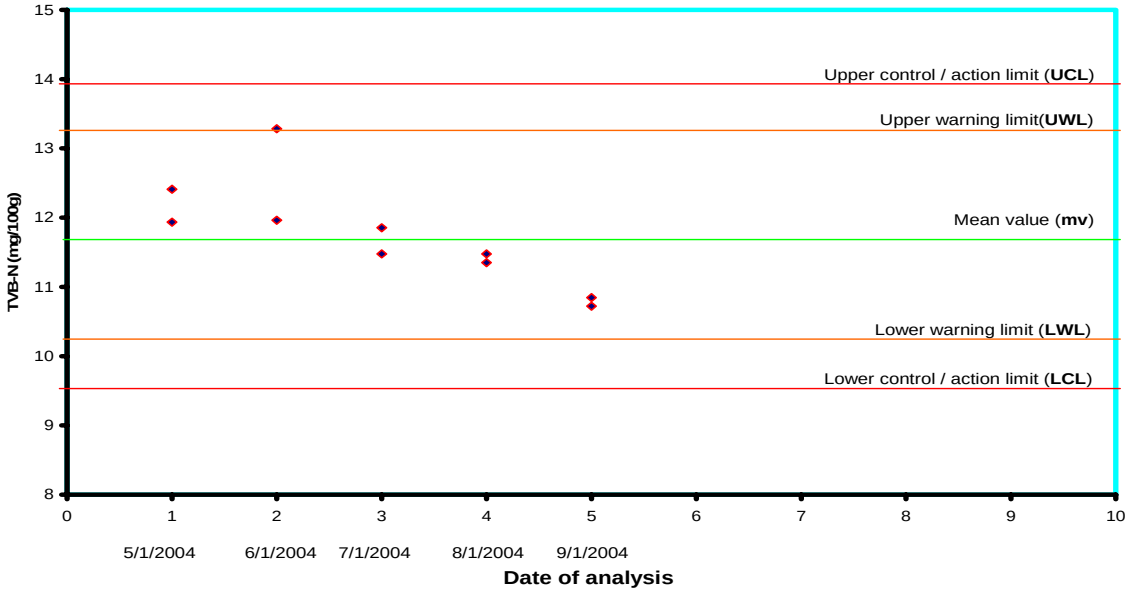


Figure 22: TVB-N control chart

4.3 Discussion

Calibration was done during the study as required by the ISO/IEC17025 standard for pH meter and analytical balance. Calibration trials were also done on working thermometers. Calibration procedures for all equipment used in the Microbiology Laboratory were compiled effectively.

As can be seen in Figures 21 and 22, all the analytical results for TPC and TVB-N laid within the acceptable regions of the charts as expected.

From the two control charts, the analytical values were not the same regardless of being from the same consignment an indication that the quality system was effective together with the personnel carrying out the analysis. However, results may remotely be expected to be equal before the charts are created.

5 CONCLUSIONS

Since the results of analysis were within the acceptable regions of the two charts, the calibration procedures are reliable and well implemented. These procedures can be combined into a calibration manual to harmonise the operations of the calibration/quality managers as a subsidiary of this project.

It is concluded that the two control charts are very sensitive quality monitoring tools since the results of the experiment open up inconsistencies, which could not be easily noticed with few uncoordinated analyses. These control charts are therefore essential when monitoring personnel and equipment performance.

From the results, we are not able to know the two real values of the TPC and TVB-N analyses. We assume the mean as the value contained but it may not be the actual value.

6 RECOMMENDATIONS

The most effective and reliable method that is recommended for the evaluations is the use of standard reference materials. For the Uganda Fisheries Laboratory, sets of three stride viols will have to be purchased from accredited suppliers. The viols will then be purchased and used continuously to perform internal quality assessment.

Ring analysis is a proposed project for all food testing laboratories in Uganda when referring to external proficiency tests done by accreditation bodies on laboratories. This is a requirement since laboratory accreditation bodies are overseas. Their monitoring work is limited since it is expensive to have them at laboratory work places at regular intervals. This then calls for a laboratory monitoring body within Uganda and other countries in similar circumstances.

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