

**PREPARATION FOR OBTAINING ACCREDITATION OF  
ANALYTICAL METHODS REGARDING QUALITY ISSUES AS  
STATED IN ISO STANDARD ISO/IEC 17025:1999.  
PROPOSAL**

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**ABSTRACT**

The present study will be a guideline for the accreditation of analytical methods regarding quality issues based specifically on ISO standard 17025 and practice steps to implement a quality system for accreditation in the fisheries laboratories in Mozambique.

It is based on the requirements of ISO standards 17025 for TVB-N analysis and Total Plate Count (TPC) in order to be a guideline for the two fisheries laboratories in Mozambique to obtain accreditation in these two analytical tests.

It includes a brief introduction of ISO standard 17025, a figure describing the quality system in general, a theoretical description of the methods (The Total Plate Count-TPC based on APHA 1992 and FDA 1995 and the TVB-N in AOAC) requirements and practical examples using the basic steps to get accreditation, like a control chart for both of them and others tests to check results obtained during practice.

It shows that accreditation can not be reached in one moment, but gradually with improvement of organisation, records, equipment, methods and personnel training all organised in a quality manual always in improvement.

## 1. INTRODUCTION

Quality systems for product analysing are now used all over the world and ISO 17025 is the standard that is most widely used for accrediting testing laboratories.

The Ministry of Fisheries in Mozambique is now finishing the construction of two new laboratories in order to improve product analysis and facilities in addition to the existing laboratories in two main cities of Mozambique. An implementation of this new system followed by audit measures will give an idea about the advantages and the disadvantages of the system but on the other hand a lot of work is necessary to train the staff and auditors. These guidelines will be useful to begin this work.

It is important to mention that TVB-N-determination is a method already implemented in both laboratories in Mozambique but Total Plate Count is only implemented in one laboratory. Both of them need time to be fully implemented, since until now they have been working with GLP (Good Laboratories Practice).

This project is based on the requirements of ISO standards 17025, and the quality system in IFL accredited by SWEDAC, to obtain accreditation for two analytical tests, TVB-N analysis and Total Plate Count (TPC), in order to be a guideline for the fisheries laboratories in Mozambique to obtain accreditation.

It includes a brief introduction of ISO standard 17025, a detailed method description of the methods, TPC and TVB-N, taking the requirements of the standard into account such as the description of the personnel background, maintenance of the equipment, driving card for the method, quality control with relevance for the control charts and a test report.

It shows that accreditation requires a lot of effort in making a quality manual where organisation, records, equipment, methods, and personnel training is described.

## 2. LITERATURE REVIEW

### 2.1 History of quality standards

The original motive for the establishment of ISO was to fulfil the need for an agreement on world standards to help the exporting industry to rationalise the international trading process.

ISO, International Organisation for Standardisation, is a non-governmental organisation established in 1947, composed by 140 national standard bodies, one from each country. The mission of ISO is to promote the development of standardisation with a view to facilitate the international exchange of goods. ISO work results in international agreements, which are published as International Standards.

The technical work is decentralised, carried out in a hierarchy of some 2850 technical committees, subcommittees and working groups. The Central Secretariat in Geneva acts to ensure the flow of documentation in all directions. In order to follow technological developments, new methods and materials, new quality and safety requirements, ISO has

established the general rule that all ISO standards should be reviewed at intervals of no more than five years. (ISO 2001)

To understand ISO standards they can be defined as documented international agreements containing technical specifications which can be used as guidelines or prototypes to ensure that products and services suit for their purposes such as a simple credit card which format was defined by an ISO standard (ISO 2001). ISO operates a special programme consisting of training seminars for the developing countries. This programme provides an important mechanism through which developing countries may accelerate the advancement of their quality assurance system.

Before ISO 17025 was established three quality model existed for food analysis laboratories in Europe (Wood 1998): The EN 45001:1989 used by National Accreditation Agencies based on the ISO/IEC Guide 25:1990, the BS 5750/ISO 9000 known as BS EN ISO 9000 in the UK and the principles of Good Laboratory Practice (GLP). It has been claimed (Vogt 2001) that ISO 9000 and EN 45001 were insufficient. According to Hoolihan (1999) and Anandavally (2001) the new standard ISO 17025 is more appropriate for laboratories than EN 45001:1989. The new standard contains all the requirements to operate a credible quality system for technically valid results.

## **2.2 Quality system**

### *2.2.1 Structure of Quality system- brief description*

According to the standard ISO17025:1999(E), the structure of the organisation and management must be clear, in order to maintain the system. The quality manager has defined responsibility and authority for ensuring that the quality system is implemented and followed at all times. The quality manager has direct access to the highest level of management.

The laboratory must implement policy and procedures for implementing corrective actions when non-conforming work or departures in the quality policy have been identified.

Quality systems shall be operational at three levels: Top management, the supervisory staffs and operating personnel (Garfield 1992).

The system shall be constructed according to the requirements of the ISO17025 and described in a Quality Assurance Manual (ISO 17025:1999)

The objective of the quality system explained in a quality manual is to clarify the relationships between different levels (staff, clients), define specific objectives, the organisation of staff, procedures, and documentation in general, in order to fulfil the standards and permit all staff members access of information. Making a quality manual is one of the fundamentals in order to get accreditation for chemical and microbiological methods. Based on the requirements of the standard, handbooks and an Internet search, a general description of the quality system and its relation to the quality manual is drawn up in Figure 1 with more focus on technical requirements.

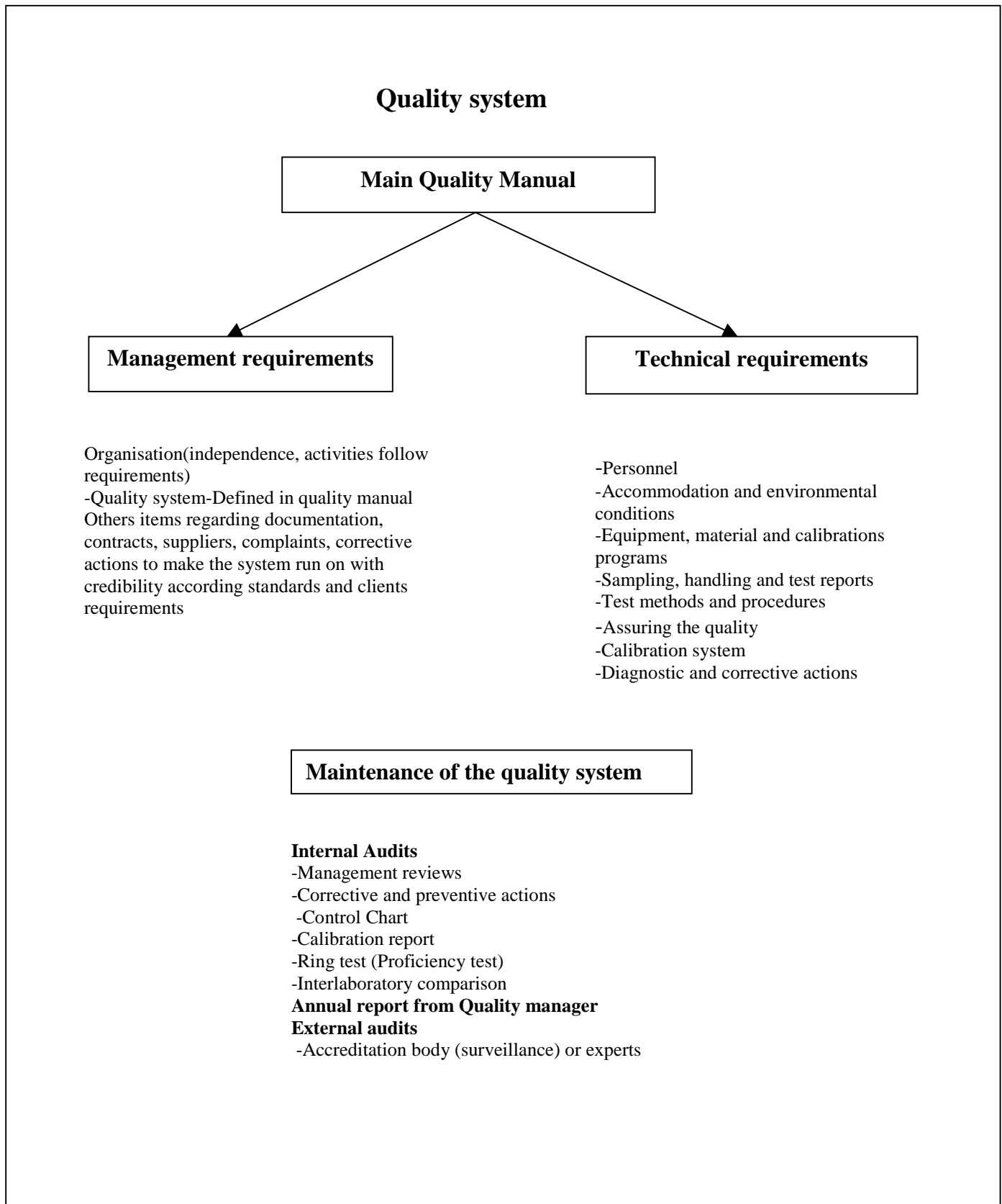


Figure 1: General Description of the quality system based on ISO 17025:1999

### 2.2.2 *Technical requirements*

#### **Personnel**

The personnel must be qualified and have enough training to correct errors in the quality system and procedures. Also, confidentiality of the report must be guaranteed to the customer (ISO 17025:1999(E), Wood 1998)

#### **Premises and environmental conditions**

The premises of laboratory are very important for the safety of the workers and the work performed such as workspace, temperature, dust, moisture, and ventilation (Garfield 1992, NMKL 1994, Wood 1998).

#### **Equipment, material and calibration**

Instructions, maintenance, and calibration of equipment and material must be documented. Defected equipment should be clearly labelled or marked as being out of service until it has been repaired as shown by calibration or tested to perform correctly. (Garfield 1992, NMKL 1994).

The laboratory must guarantee the quality and purity of chemical reagents, solvents, gases, primary reference and secondary (house) standards, standards solutions, purified water, and volumetric glassware used in analysis. The laboratory shall have calibration programme and the instruments must be calibrated and checked to establish that it meets the laboratory's specification requirements and complies with the relevant standard specification. (Garfield 1992)

#### **Sample handling and test reports**

The laboratory must have a system that documents handling of samples, which guarantees traceability (Garfield 1992, ISO17025: 1999(E))

The results are presented in a test report, which must include

- Name and address of the laboratory
- A unique serial number identifying each page of the report
- Name and address of the client
- Identification of the method used
- Description of the item
- Date of item receipt, examination, and analysis as appropriate
- Description of sample preparation
- All measurements, examinations, and derived results supported by tables, graphs, sketches, charts, and photographs.
- A statement of measurement uncertainty.
- A statement as to whether or not the sample complies with any requirement against which it was assessed

The name, function, and signature of a person that authorise the test report.

## Test methods and procedures

The laboratory shall use methods and procedures according to the request and the requirement of the client:

- Standardised methods like in Association of Official Analytical Chemists.
- In case of usage of non-standard methods, the procedure must be validated.

(Garfield 1992)

### 2.2.3 Maintenance of the quality system

A quality system can be maintained by internal audits, annual reports and external audits but the basis of the quality system is the internal audit due to the frequency and measures to prevent, solve, and correct.

It is important to keep all the records in order and easy to locate to understand the changes and improvements and to facilitate any audit of the quality system.

A good maintenance of the system can be reflected in the results particularly in inter-laboratory comparison and proficiency tests.

## Assuring the quality of test results

The laboratory must have quality control procedures for monitoring the validity of tests. Internal control can be conducted by:

- *Control chart.*  
A control chart is a diagram to evaluate whether a process is within statistically acceptable limits. When constructing a control chart a control sample is measured 15-20 times. The average value and the standard deviation (Sd) are calculated. The control chart is set up with five horizontal lines; one presenting the mean value, two lines indicating  $\pm 2Sd$  (warning limit) and two lines indicating  $\pm 3Sd$  (action limit). A control sample can either be a reference material or an internally produced sample. It is important that the control sample is homogeneous and has a similar composition as the samples that will be analysed (NMKL 1996).
- *Inter-laboratory comparisons and proficiency testing.*  
Information about performance of a laboratory can be obtained from inter-laboratory comparisons, which also gives indications of the competence of personnel. (SWEDAC 1999). For a list of websites on further information about inter-laboratory tests, see Appendix IV.
- *Reference material.*  
Using certified reference materials is one way to tackle unknown errors. Reference material can be expensive and certified reference materials sufficiently similar to the analytical task at hand may not always be available. (Wood 1998)

### 3. METHODS

In this project the main task was to get acquainted with the standard ISO 17025:1999 and the general requirements for the competence of testing and calibration laboratories in order to get an accreditation for a laboratory.

To keep the project within the given timeframe it was decided to concentrate on the technical requirements of the ISO standard. Main documents necessary for method accreditation were made. These are; a method description, description of the equipment used, skill and competence of the personnel, 'driving card for the method' and a test report (Appendix 1). In addition supplementary guidelines published to assist laboratories in the accreditation process were studied.

A method description on TVB-N and TPC was made using appropriate methods. Both methods are standard methods as recommended by the ISO standard.

The standard requires the laboratory to have instructions on the use and operation of all relevant equipment and on the handling and preparation of items for testing. Consequently a document was made for the equipment used in the TVB-N method.

Competence and training of the personnel is one of the most important factors to guarantee. A document was made describing qualifications, training and experience of each employee. A 'driving card' is issued when a person has received enough training to operate a specific method.

The ISO standard also requires the results of each test carried out by a laboratory to be accurately, clearly, unambiguously and objectively reported, and in accordance with any specific instructions in the test. These instructions are given in the 'test report' in Appendix 1.

Last but not least the internal control of methods was studied. Internal control is very important in assuring the quality of test results.

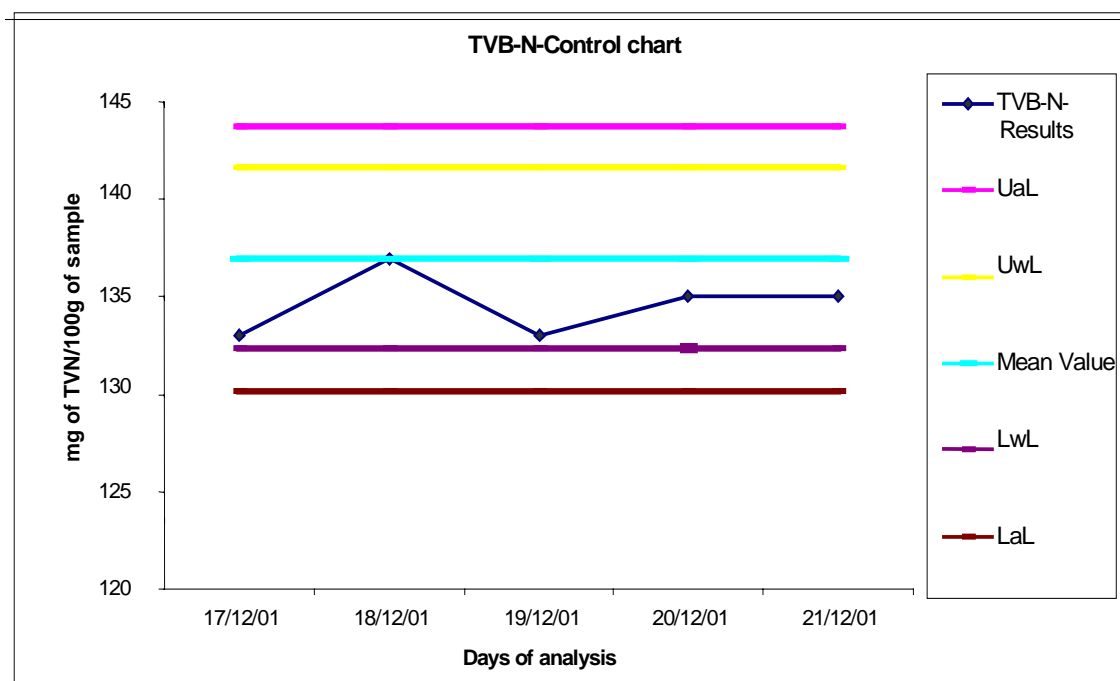
## 4. PRACTICAL WORK ON INTERNAL CONTROL OF TVB AND TPC

### 4.1 Quality control procedures for TVB-N determination

Internal control was conducted through control chart and recovery test.

#### 4.1.1 Control chart

A control sample was run five times. The results are shown in the control chart. (Figure 2) All the samples are within the upper and lower warning limits. Results (raw data) are shown in Appendix II.



UwL upper warning limit	LwL lower warning limit
UaL upper action limit	LaL Lower action limit

Figure 2: Control Chart for TVB-N determination in Fishmeal (control sample) based on 20 TVB-N determinations from the staff of IFL laboratory. To check the ability of the analyst and the performance of the procedures, 5 TVB-N determinations were made in this practical example.

#### 4.1.2 Recovery test

A recovery test is one way to see if a method is running properly. Consequently a recovery test was done according to the TVB-N method description. The results are shown in Appendix III. The result shows data between 95.1% and 99.1%. The limits



however should be around 99.5%-100.5% and the difference can be because lack of experience.

## 4.2 Quality control procedures for TPC determination

Internal control was conducted through control chart and additional trials.

### 4.2.1 Control chart

A control sample was run eleven times. The results are shown in the control chart. (Figure 3) All the samples are within the upper and lower warning limits. Results (raw data) are shown in Appendix III.

The results in Figure 3 show that all points lie within the upper control limits (UCL) and the lower control limits (LCL), which means that there is only a common cause of variation. However, it is necessary to remember that the (UCL) and (LCL) were calculated on the basis of my own results and my own standard deviation. This means that the operator a source of error. Consequently, the best way to construct a control chart for one laboratory is to involve all the people working with the method to have a mean value approximately according the normal procedures, and to minimise the risk of having the limits too narrow.

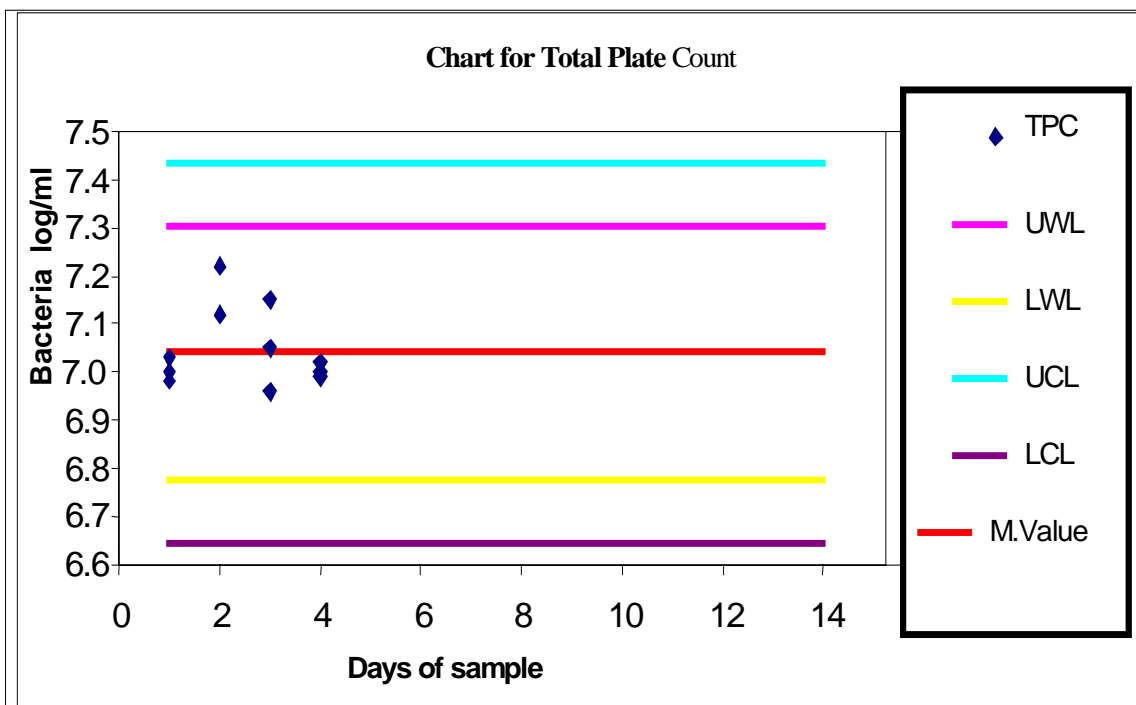


Figure 3: Quality control chart for Total Plate Count made on the basis of 11 samples grouped in 4 days to be a control chart for the next evaluations.

It is also interesting to consider the locations of the data points on different days (close in 2 days of the practice and dispersed on other 2 days). This is due to the human factor in

this case. This means that before making a control chart it is necessary to have enough practice with the method (Total Plate Count). The human factor aside, other factors that can influence the dispersion of data points outside the limits must be considered. One way to do that is grouping the plates in subgroups according to factors such as temperature and time of incubation.

Although all the results are within the warning limits there are some points close to the outer boundary. This means that some technical procedures, for example the pipetting techniques, need to be revised and improved. To verify the limits determined in this manner, the control chart should be routinely used in the next months and the results of different staff members compared.

#### *4.2.2 Additional trial for Total Plate Count*

A trial for TPC is an internal control of the method, and E.coli is used due to the easy growth in that medium (see procedure in TPC method description, in APPENDIX 1). The objective is to evaluate the performance of the analyst in making dilutions and passages and recovering the original number of bacteria. No exact rules are available for how much should be recovered. The number however shows the performance of the method and the analyst and should be as closed to the initial number of bacteria as possible. The recovered numbers in this experiment were 9900, 12050 and 11300 (cells) for an initial number of 13000 cells. The actual results are shown in Appendix III.

## 5. CONCLUSIONS

- This project shows that internal/external training and practice are very important if accomplished with internal and external audits.
- Periodical participation in inter-laboratory tests and proficiency tests are very important to test the performance of our own laboratory but before starting, it will be better to do some internal audits first.
- A control chart for both methods was a good example of how quality control of the personnel, the method and the procedures in general can be achieved.
- In test comparisons the human factor, environment, apparatus, simplicity of the method and sampling of the item to test, plays an important role in the results.
- A quality manual is a document specific and very important for each laboratory and must address the situation of: premises, staff, organisation, national standards, methods, equipment, quality system, requests and always kept up to date according to advanced models.

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## APPENDIX I: SIX DOCUMENTS WHICH CONSTITUTES A PART OF A QUALITY MANUAL

According to ISO 17025 a detailed description of the methods must be kept in a quality manual.

### TVB-N. A method description based on AOAC and ISO 17025

**Title**-TVB-N-Determination in fish<sup>1</sup>

<b>TVB-N in fish</b>	<b>Method number</b>
<b>Responsible for the method-</b>	<b>Version:</b>
<b>Responsible for Struer Apparatus</b>	<b>Last versions in</b>
<b>Reference -AOAC 15th ed. 1990 920.03</b>	<b>Last revision</b>
<b>Transcription and translation by-</b>	<b>Version number-</b>
<b>Accepted by-</b>	<b>Page-01 of ( )</b>
<b>Implementation date-</b>	<b>Audited by</b>

- **Method no.** Ghb-e-AM-906
- **Implementation date:**01/06/00
- **Title:** TVB-N in fish
- **Reference:** AOAC 15<sup>th</sup> ed. 1990 920.03
- **Limits of Acceptance :**
  - a. measurement range for TVB-N in fish flesh 2mgN/100g – 120mgN/100g
  - b. uncertainty NH<sub>3</sub>-N: 0.003%
- **Scope:** TVB-N (total volatile base Nitrogen) is a combination of trimethylamine (TMA), dimethylamine (DMA), formaldehyde (FA,) Ammonia, and other volatile nitrogen substances.
- **Instruments and material.**

<b>Instruments</b>	Analytical balance with 0.01g accuracy Struer-TVN steam distillation apparatus with appropriate distillation bottles.
<b>Material</b>	Boric acid (10g/l) H <sub>2</sub> SO <sub>4</sub> – 0.1000 standardisation H <sub>2</sub> SO <sub>4</sub> – 0.0300 standardisation NaOH – 0.1000 standardisation Mixed indicator bromocresol green/methyl red Distilled and deionized water (free of CO <sub>2</sub> ) Magnesium oxide (MgO pondorosum) Potassium Hydrogen Phthalate (C <sub>8</sub> H <sub>5</sub> KO <sub>4</sub> , Merck 4876) Phenolthalen indicator (1% in ethanol)

<sup>1</sup> A draft of a document in a quality manual

### Quality control and Security

- Recovery test must be performed every month  
*Material:*  $(\text{NH}_4)_2 \text{SO}_4$  (Ammonium Sulphate), (21.09% N) dried before use for 4 hours at  $103^\circ\text{C}$   
*Procedure:* Weigh out 0.15 g of Ammonium Sulphate in a boiling bottle, add 10 ml of NaOH (10%) and 100ml of water. Place the boiling bottle in Struer apparatus and boil for 15 minutes. Vapour is collected in 50 ml of Boric acid. The content of the collection bottle is titrated from green colour to grey colour.  
 The recovery test should be between 99.5%-100.5%. The difference between duplicates shall not be more than 0.5 mgN/100g when TVB-N is 2-40 mgN/100g and not more than 1 mgN/100g when TVB-N is  $> 40$  mgN/100g.
- Control chart is used to monitor the method and is done every time as sample is measured.
- It is important for the laboratory to participate in a proficiency test at least once a year.

The boiling bottle used must always be intact and the same goes for the pipette used. It is important to use eye protection and gloves at all times.

### Maintenance and cleaning of Instruments

Wipe the instrument and surroundings with a moist cloth after each use. The distillation apparatus shall be cleaned as needed by distilling 25 ml of water + 25 ml of Acetic acid for 15 to 30 min. Rinse the system afterwards by distilling deionize water 2 or 3 times for 10 min. Check for absence of acid by distilling a blank sample.

In case of suspicion of altered steam flow in the instrument, measure the time it takes to distil 100 ml into a measuring cylinder.

### Procedure

#### a) Preparation and standardisation of 0.1000 N Sodium Hydroxide (NaOH)

**Materials:** Sodium Hydroxide,  
 deionised water,  
 Potassium Hydrogen Phthalate ( $\text{KHC}_8\text{H}_8\text{O}_4$ )  
 Phenolphthalein indicator (1% ethanol solution)

**Preparation:** Weigh 20.4g of NaOH into a 5 L volumetric bottle and fill to the mark.

**Standardisation:** (in triplicate)

1. Weigh exactly 0.8000 g of  $\text{KHC}_8\text{H}_8\text{O}_4$ , transfer into a 250 ml Erlenmeyer bottle and add 40 ml of  $\text{H}_2\text{O}$ . (V1)
2. Add 3-4 drops of indicator (phenolphthalein) and titrate with the NaOH solution (remove 100 ml from the original volumetric bottle for the titration) until the first colour change (formation of pink colour)
3. Blank sample. Put 40 ml of water with 3-4 drops of phenolphthalein into another Erlenmeyer bottle and titrate until endpoint (no more than 1 drop). Deduct that result from the result in step 2. (V2)

Calculations of normality =  $\frac{\text{g KHC}_8\text{H}_8\text{O}_4 * 1000}{\text{ml NaOH } 204.229}$

5. Standardise exactly to 0.1000 N: The strength of NaOH should be just above 0.1N since we weighed slightly more NaOH than needed. To adjust the solution exactly to 0.1000N, it is necessary to add some water according to the following formula.  $V_1 = V_2 * N_2/N_1$ , where  $N_2$  and  $V_2$  are strength and volume of the NaOH solution we prepared and  $V_1$  is the final volume needed in order to dilute the solution sufficiently to get exactly 0.1000N NaOH ( $N_1$ ).

Reference: AOAC (1990), 15 ed. Method no 936.16

**b) Preparation and standardisation of 0.1000 N H<sub>2</sub>SO<sub>4</sub> solution**

**Materials:** Sulphuric Acid (96-98% H<sub>2</sub>SO<sub>4</sub>, extra pure, e.g. Merck 713), distilled or deionized water (free of CO<sub>2</sub>),

**Preparation**

1. Weigh about 30.4 g of H<sub>2</sub>SO<sub>4</sub>, into a volumetric flask and fill to 6 l.
  2. Standardisation the solution; 20 ml of the acid are titrated with standardised 0.1000 NaOH. Indicator is methyl.
- Calculations: Strength of H<sub>2</sub>SO<sub>4</sub> = ml NaOH \* N NaOH / 20 ml.

Reference: AOAC (1990), 15 ed. Method no 936.16

**c) Standardisation of 0.0300 N H<sub>2</sub>SO<sub>4</sub> solution with a standardised 0.1000 N NaOH solution.**

**Material:** Sulphuric Acid (96-98% H<sub>2</sub>SO<sub>4</sub>, extra pure, e.g. Merck 713), distilled and deionized water (free of CO<sub>2</sub>),

**Standardisation.**

1. Weigh about 1.6 g of H<sub>2</sub>SO<sub>4</sub>, into a volumetric flask and fill to 1 l.
  2. Standardise the solution; 10 ml of 0.1000 NaOH are titrate with the acid. Indicator is phenolphthalein.
- Calculations: Strength of H<sub>2</sub>SO<sub>4</sub> = N NaOH \* 10 ml / ml H<sub>2</sub>SO<sub>4</sub>.

Reference: AOAC (1990), 15 ed. Method no 936.1

**Boric Acid. (1% with bromocresol green/methyl red indicator)**

Dissolve 50 g of Boric Acid in ca. 4 l of deionized water. Add 50 ml of bromocresol green (100 mg in 100 ml of ethanol) and 35 ml of methyl red (100 mg in 100 ml ethanol). Dilute to 5 L and mix. Put 25 ml of this boric acid solution into a collection bottle plus 100 ml of water. If the solution in the bottle still red then titrate with 0.10 N NaOH until a neutral colour is reached.

The quantity of 1.0 N NaOH that is needed (to add to the Boric acid solution) is calculated according to the formula: ml 1.0 N NaOH = ml titrant \*40

A grey colour should always be obtained by mixing 25 ml of the boric acid solution and 100 ml of water.



**e) Measurement of TVB-N in fish flesh**

Always work with double samples.

About 10 g of sample are weighed with 0.01 g accuracy and straight into the distillation bottle, add about 3 g of MgO (magnesium oxide) and 100 ml of deionized water (add the water in small portions to avoid lumps of fish).

Arrange distillation flask in the distillation apparatus. A collection flask with 50 ml of Boric acid is placed under the tube from the cooling coil. Take care that the end of the tube is immersed in the boric acid. Start distilling and continue for 15 min.

**Titration**

The contents of the collection flask are titrated from green to grey with 0.0300 N H<sub>2</sub>SO<sub>4</sub>.

**Calculations**

$$\text{mg N/100g} = [(\text{ml} * \text{N} * 14.01) / \text{g}] * 100$$

ml = ml of H<sub>2</sub>SO<sub>4</sub> titrated

N = strength of H<sub>2</sub>SO<sub>4</sub>

g = weight of sample (10g)

14.01 molecular weight of Nitrogen

## Equipment maintenance and calibration

The following sheet is a detailed explanation of how to use, maintain, and control the equipment.

### Name -Struer Apparatus for measurement of TVB-N (Steam distillation)<sup>2</sup>

- 1-Responsible for the instrument-
- 2-Reference number and Serial number-
- 3-Manufacturers name
- 4-Name, address and telephone number of maintainer
- 5-Location of manual/instruction -
- 6-Location of spare parts -

### Operating instructions

When switching on the equipment, make sure the cooling water is turned on and the water container for distillation water kettle is filled. Before and after measuring, clean the apparatus by distilling water for approximately 5 minutes. Then the equipment is ready to use.

Any accident must be reported immediately to the responsible party.

New personnel can not use the equipment without reading the instructions and must be under supervision in the first run of samples.

### Control and maintenance plan

Wipe the instrument and surroundings with a moist cloth after each use. The distillation apparatus shall be cleaned as needed by distilling 25 ml of water + 25 ml of Acidic acid for 15 to 30 min. Rinse the system afterwards by distilling deionize water 2 or 3 times for 10 min. Check for absence of acid by distilling a blank sample.

In case of suspicion of altered steam flow in the instrument, measure the time it takes to distil 100 ml into a measuring cylinder..

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<sup>2</sup> A draft of a document in a quality manual

## TPC (Total Plate Count)

Detailed method description according to requirements on ISO 17025

**Title**-TPC determination<sup>3</sup>

**TPC-Pour Plate method in fish products**  
**Responsible for the method-**  
**Responsible for Incubators and autoclave-**  
**Reference -** APHA 1992 3rd ed., FDA 1995  
**Transcription and translation by-**  
**Accepted by-**  
**Implementation date** -

**Method number**  
**Version:**  
**Last versions in**  
**Last revision**  
**Version number-01**  
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### Procedure

**1. Sample preparation** -When examining solid samples like fish a 1/10 dilution is made by mincing and mixing 25 g of mince with 225 g of dilution buffer.

**2. Labelling.** Label all petri plates, and tubes and bottles where necessary, with the sample number, dilution, date, and any other desired information. The bench area should be cleaned and sanitised, and all possible sources of contamination removed or reduced to a minimal level.

**3. Dilutions and plating.** Two more plates per dilution should be employed. The first pair of plates are inoculated with 2\*1 ml (1/10) and the next pair of plates with 2\*0.1 ml (1/100). Higher dilutions might be required. Do not insert the pipette more than 2.5 cm below the surface of the sample. The pipette should be emptied in the diluent (phosphate buffered water or 0.1% peptone water) by letting the column drain from the graduation mark to the rest point of the liquid in the tip of the pipette within 2 to 4 sec. Promptly and gently blow out the last drop when pipetting the undiluted sample, or when using a pipette designed to blow out the last drop.

Vigorously shake all dilutions 25 times in a 30-cm arc for 7-sec. Optionally, a mechanical shaker may be used to shake the dilution blanks for 15 sec.

If a pipette becomes contaminated before completing transfers, replace it with a sterile pipette.

Use a separate sterile pipette for transfers from each dilution. Dilution blanks should be at room temperature (15°C to 25°C) when used.

**4. Melting and tempering media.** Melt agar media in flowing steam or boiling water, avoiding prolonged exposure to high temperatures. Temper melted media promptly and maintain between 44°C and 46°C until used. Set a thermometer into water or medium in a separate container similar to that used for the test medium; this temperature control medium must have been exposed to the same heating and cooling as the test medium.

<sup>3</sup> A draft of a document in a quality manual

After inoculation, melted plate count agar (45°C) with 0.5% NaCl is poured on the plates and the content is mixed gently.

**5. Incubation.** All plates are incubated inverted at 35  $\pm$  0.5 °C for 48  $\pm$  3 hrs (or any other temperature required). Do not stack more than 4 plates on top of each other.

**6. Counting the plates.** Colonies are usually counted over light with a double magnification in a Quebec colony counter.

Colonies can also be counted by inverting the plates and mark each colony with a marker. Plates from the dilution showing colony numbers 25 to 250 are chosen for counting.

If two plates are used per dilution count colonies on both plates, find the mean and multiply with the corresponding dilution factor.

According to the FDA it is sufficient to use 2 significant numbers. If no plates contain more than 25 colonies, count the number of colonies in the lowest dilution used.

If no colonies are found in the lowest dilution (1/10) results are reported as TPC < 10/g. If the number of colonies exceed 250 in the highest dilution, an area of 10cm<sup>2</sup> (10 squares) is counted, divided by 10 and multiplied with the total area of the plate (56 cm<sup>2</sup> for ordinary plastic plates)

### Precautions and limitations

Colony count methods provide an estimate of the number of viable micro-organisms in food according to the medium employed and the time and temperature of incubation. Microbial cells often occur as clumps or groups in foods. Whereas shaking samples and dilutions may uniformly distribute the clumps of bacteria, this may not completely disrupt the clumps themselves. Mixing the initial dilution in a mechanical blender may provide better breakdown of the clumps. However, this does not ensure that the micro-organisms will be distributed as single cells. Consequently, each colony that appears on the agar plates can arise from a clump of cells or from a single cell and should be referred to as a colony-forming unit (CFU).

### Internal control of the method

#### Additional trials for TPC

Fresh strain of *Escherichia coli* from DSMZ in Germany was used. Culture from nutrient agar slant was inoculated into nutrient broth and grown at 35°C for 1 day. From that broth one loop full was inoculated into fresh nutrient broth and incubated for 2 days. The culture was diluted and grown on Plate Count Agar (pour plate) at 35°C for 1 day. The density found was ca.  $1.3 \times 10^8$ /ml. The strain was diluted as follows: 1 ml was put in 99 g of buffer ( $1.3 \times 10^6$ /ml) and from that, 1ml was mixed with 100 g of minced shrimp. The number of *E.coli* should therefore be  $1.3 \times 10^4$  per g of that mixture. Then 13000 cells /g of shrimp were into 25 g added to 225g of buffer and blended in a stomacher; 1 ml should give ca. 13000 cells per g shrimp. That solution was mixed with PCA (pour-plate) and incubated at 30° -C for 2 days. The same shrimp sample was used for the tests and it contained prior to inoculation 1000 cells/g.

## Employee identification chart according to the requirements of ISO 17025

It is important to know the qualification of the personnel and their background to make sure that they are able to solve, prevent and correct any situation or occurrence, if they are able to do, interpret and perform tests, and if they need some training or refresher courses.

### Personal information<sup>4</sup>

Name -

Other occupation-

Former employment

Position-

Date of employment

Education-

Signature-

List of retraining

### ‘Driving card’

It is important to have a control like this to guarantee the credibility of the results and also to specify the analysis for each worker according his capacity.

### Driving card

Name

Category - Quality inspector level

Code of Analysis	Analysis	Duration of training	Date of accomplishment	Signature of trainee	Signature of trainer
AM-906	TVB-N	2 weeks	20-12-01		
AB-00301	TPC	3weeks	13-12-01		

<sup>4</sup> A draft of a document in a quality manual

<sup>5</sup>

## Test Report

It is important to follow the requirements of the ISO 17025 regarding the information are allowed on the test report.

### Test report<sup>6</sup>

-Analytical Division-Chemical Laboratory

Test Report serial number

Page 1 of ( )

Client -Address and contact

Method-TVB-N-903-06

Sample type-

Date of sample reception-

Date of test-

### Results

Sample description	Sample Number	mg of TVB-N/100mg Sample	Date of test
	1		17/12/01
	2		18/12/01
	3		19/12/01
	4		20/12/01
	5		21/12/01

Test report authorised and supervised by

Name, function and signature-

Date-

Laboratory contact address in detail-

NB: The results above described correspond only to the samples analysed and any reproduction of this Test Report must contain all document under written approval of this Laboratory.

<sup>6</sup> A draft of a document in a quality manual

## APPENDIX II: RESULTS FROM PRACTICAL WORK FOR TVB-N (CONTROL CHART AND RECOVERY TEST)

Table 1: Results from TVB-N determination on a control sample to make a control chart

Person	Date	TVB-N
ij	04/10/00	0.138
ij	04/10/00	0.136
ij	05/10/00	0.138
ij	05/10/00	0.141
ij	06/10/00	0.138
ij	06/10/00	0.138
ij	11/10/00	0.136
ij	11/10/00	0.136
ij	18/10/00	0.132
ij	18/10/00	0.139
ij	20/10/00	0.135
ij	20/10/00	0.137
ij	21/10/00	0.133
ij	27/10/00	0.137
ij	30/10/00	0.138
ij	30/10/00	0.140
ij	03/11/00	0.136
ij	03/11/00	0.138
ij	06/11/00	0.140
gg	13/11/00	0.135
nv	17/12/01	0.133
nv	18/12/01	0.137
nv	19/12/01	0.133
nv	20/12/01	0.135
nv	21/12/01	0.135

NB: last five determinations were filled in a control chart

Table 2: Calculation of Mean Value, Upper, and Lower warning Limits and Upper and Lower action Limits for control chart

Designation	Calculation	Results
Mean Value(MV)	$\sum x/n$	0.137
Standard deviation	$=\sqrt{\sum(x-MV)^2/n-1}$ 0.0023	0.0023
Upper/Lower action Limits	$MV\pm 3xsd$	0.1438/0.1302
Upper/Lower Warning Limits	$MV\pm 2xsd$	0.1416/0.1324

Table 3: Recovery test for Struer apparatus

Weight ammonium sulphate	of	ml of titrate(Sulphuric acid)	mg N/100g	of	% of recovery (Limits-99.5- 100.5)	Done by	Date
0.1517		23.15	20.71		99.1	NV	17/12/01
0.1505		22.8	20.56		98.4	NV	18/12/01
0.1502		22	19.88		95.1	NV	19/12/01
0.1502		22.35	20.42		96.6	NV	20/12/01
0.1502		22.7	20.42		98.1	NV	20/12/01
0.1500		22.7	20.7		98.3	NV	21/12/01



### APPENDIX III -RESULTS FROM PRACTICAL WORK FOR TPC (CONTROL CHART AND ADDITIONAL TRIAL)

Table 1: Calculation of Mean Value and standard deviation for control chart

Analysis n°	Date	Log Bacteria/ml(x)	(x-MV)	(x-MV) <sup>2</sup>	Done by
1	26/11/01	6.98	-0.06	0.0036	NV
1	26/11/01	7.00	-0.04	0.0016	NV
1	26/11/01	7.03	-0.01	0.0001	NV
2	3/12/01	7.22	0.18	0.0324	NV
2	3/12/01	7.12	0.08	0.0064	NV
2	5/12/01	6.96	-0.08	0.0064	NV
3	5/12/01	7.15	0.11	0.0121	NV
3	5/12/01	7.05	0.01	0.0001	NV
4	10/11/01	7.02	-0.02	0.0004	NV
4	10/11/01	6.99	-0.05	0.0025	NV
4	10/11/01	7.00	-0.04	0.0016	NV
n=11		Ex=77.52		E(x-MV) <sup>2</sup> =0.1762	
Mean Value (MV)		Ex/n=7.04			

Table 2: Calculation of Upper and Lower warning Limits and Upper and Lower action Limits for control chart

Designation	Calculation	Result
Standard deviation	$=\sqrt{E(x-MV)^2/n-1}=0.132$	0.132
Upper/Lower action Limits	MV $\pm$ 3xsd	7.304/6.776
Upper/Lower Warning Limits	MV $\pm$ 2xsd	7.436/6.644

Table 3: Aerobic Plate Count (Trial for TPC). Count of last dilutions ( $10^{-6}$ ) to check density for additional trial

Dilution	Bacterial count(CFU)	Density
$10^{-6}$	132	$=1.3 \times 10^{-8}$
$10^{-6}$	134	$=1.3 \times 10^{-8}$

Table 4: Counts of dilutions  $10^{-2}$  and  $10^{-3}$  for recover bacterial cells for additional trial

Plaques Dilutions	Sample1	Sample1	Average	Sample2	Sample2	Average	Sample3	Sample3	Average
$10^{-2}$	104	114	109	139	122	130,5	118	128	123
$10^{-3}$	9	16	12,5	10	9	9,5	14	11	12,5

NB: Only the averages of dilution  $10^{-2}$  were used to calculate

Table 8. Number of recovered cells with the initial number of 13000 cells in the additional trial

Samples	Recovered cells	Final after subtracting 1000Cells
Sample1	10900	9900
Sample2	13050	12050
Sample 3	12300	11300

NB: All recovers were subtracted by 1000cells/g number of bacteria in minced shrimp prior to inoculation

## **APPENDIX IV: LIST OF WEB SITES OF INTEREST FOR PROFICIENCY TESTS**

- EPTIS-European Information System on Proficiency Testing Schemes  
[www.eptis.bam.de](http://www.eptis.bam.de)
- AIHA American Industrial Hygiene Association ,United States [www.aiha.org](http://www.aiha.org)
- AOAC International, United States  
[www.astm.org](http://www.astm.org)
- BAM-Bundesanstalt fur Materialforschung und- Prufung, Germany  
[www.bam.de](http://www.bam.de)
- CTS-Collaborative Testing Services ,United Sttes  
[www.collaborativetesting.com](http://www.collaborativetesting.com)
- NATA-National Association of Testing Authorities, Australia  
[www.nata.asn.au/](http://www.nata.asn.au/)
- RELACRE-Associacao de Laboratórios Acreditados de Portugal:  
[www.relacre.pt](http://www.relacre.pt)
- APLAC-Asia Pacific Laboratory Accreditation Cooperation:  
[www.ianz.govt.nz/aplac/](http://www.ianz.govt.nz/aplac/)
- EA-European Co-operation for Accreditation:  
[www.european-accreditation.org/](http://www.european-accreditation.org/)
- EURACHEM  
[www.eurachem.bam.de](http://www.eurachem.bam.de)
- EUROLAB-European Federation of National associations of Measurement ,Testing and Laboratories  
[www.eurolab.org](http://www.eurolab.org)
- ILAC-International Laboratory Accreditation Cooperation  
[www.ilac.org/](http://www.ilac.org/)