Accreditation as stated in ISO/IEC 17025: 2005 of analytical method for determination of total volatile basic nitrogen in fish

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Abstract

This document sets out guidelines for the implementation of internal quality control (IQC) in analytical laboratories based specifically on ISO standard 17025 for determination of total volatile basic nitrogen TVB-N according to **Commission Regulation (EC) No 2074/2005**. The values of determination results of repeatability lie between the UCL and the LCL indicating that the analysts were competent and the repeatability values (r values) and Relative Standard Deviation percent (RSD %) were calculated. The laboratory was participated in international proficiency tests PT with international proficiency provider organizations for determination of total volatile basic nitrogen of canned fish to achieve the requirement of accreditation to ISO 17025:2005. The assigned value (x_a) (was 37.4 mg/100g of sample) and the laboratory result was 33.3mg/100g of sample. Z-scores are considered satisfactory if $|z| \leq 2$ (-1.7). The Laboratory used accuracy and precision control charts to determine if the measurement system process is in control and whether the results generated by the measurement system are acceptable.

Key words: *quality control, ISO standard 17025, TVB-N, accuracy and precision.*

Introduction

'Quality' has become very important. All successful organizations want to be associated with the word 'Quality'. So, what is Quality? Quality is fitness for purpose, the Degree to which a set of inherent characteristics fulfils requirements. This clearly indicates that achieving quality means fulfilling requirements **(FAO, 2011).**

EGAC is Internationally recognized by both the International Laboratory Accreditation Cooperation "ILAC" and the International Accreditation Forum "IAF". Any Certificate issued by an EGAC accredited conformity assessment body is recognized in most countries of the world. The Egyptian Accreditation Council, EGAC is headed by the Minister of Trade & Industry and is established by the Presidential Decree number 312/1996 and reorganized by the Presidential Decree number 248/2006 as the sole national body for the assessment and accreditation of conformity assessment bodies in Egypt performing testing/ calibration Laboratories, inspection and certification of products & systems as well as personnel.

ISO/IEC 17025 was prepared by the ISO Committee on conformity assessment (CASCO). It was circulated for voting to the national bodies of both ISO and IEC, and was approved by both organizations. Clause 4 specifies the requirements for sound management. Clause 5 specifies the requirements for technical competence for the type of tests and/or calibrations the laboratory undertakes.

The schedule format should typically define the laboratory's accreditation in terms of:

(i) The range of products, materials or sample types tested or analysed;

(ii) The tests or analyses (or types of tests or analyses) carried out;

(iii) The specification or method/equipment/ technique used;

(iv) The concentration range and accuracy/ precision as appropriate **(UKAS, 2000).**

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

Thompson' and Wood (1995) considered the Internal Quality Control IQC is one of a number of concerted measures that analytical chemists can take to ensure that the data produced in the laboratory are fit for their intended purpose. In practice, fitness for purpose is determined by a comparison of the accuracy achieved in a laboratory at a given time with a required level of accuracy. IQC is undertaken by the inclusion of particular reference materials, here called "control materials", into the analytical sequence and by duplicate analysis. IQC is a final check of the correct execution of all of the procedures (including calibration) that are prescribed in the analytical protocol and all of the other quality assurance measures that underlie good analytical practice. Ideally both the control materials and those used to create the calibration should be traceable to appropriate certified reference materials. When this is not possible, control materials should be traceable at least to a material of guaranteed purity or other well characterised material. **Riquixo (1998)** evaluated the suitable chemical methods for seafood products and investigated with respect to their accuracy and suitability for determination of additive in seafood products, freshness, nutritional value and scombroid poisoning in fish in Mozambique. **King (2001)** suggested that the new laboratory accreditation standard, ISO/IEC 17025, reflects current thinking on good measurement practice by requiring more explicit and more demanding attention to a number of activities. These include client interactions, method validation, traceability, and measurement uncertainty. Since the publication of the standard in 1999 there has been extensive debate about its interpretation. It is the author's view that if good quality practices are already in place and if the new requirements are introduced in a manner that is fit for purpose, the additional work required to comply with the new requirements can be expected to be modest. The issues addressed include the benefits, specifying the analytical requirement, establishing traceability, evaluating the uncertainty and reporting the information. **Velho (2001)** studied the 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. **Dobecki (2002)** reported that in order to achieve and maintain an accreditation, the requirements laid down in a new standard **(ISO/ IEC 17025)** should be met by laboratories. This paper presents the interpretation and some comments on the aforesaid requirements in the specific area of activities carried out by industrial hygiene laboratories. In particular, the author refers to the management requirements (organization, quality system, document control and service to the client, corrective and preventive actions) and technical competence (personnel, facilities, equipment, tests and sampling methods, quality control, reporting etc. **Sithisarankul *et al.*, (2002)** analyzed the current situation of laboratory accreditation (LA) in Thailand, especially on occupational and environmental health. The study integrated both quantitative and qualitative approaches. Qualitative research revealed that international standard for laboratory accreditation for both testing laboratory and calibration laboratory was ISO/IEC Guide 25, which has been currently revised to be **ISO/IEC 17025**.

Stephen (2003) conducted a study and practice of laboratory equipment calibration together with TPC and TVB-N methods of analysis according to ISO/IEC 17025 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. **Panadda Silva (2007)** recognized the laboratory accreditation as an efficient tool for putting in place a quality system for achiev-

ing continuous improvement in laboratory services in a sustainable fashion. The laboratory accreditation system is important for the acceptance of test results nationally and internationally.

Total Volatile Basic Nitrogen (TVB-N) determination is a method already implemented in Food Hygiene Department laboratories, Animal Health Research Institute, Egypt and has known to be the most common chemical parameter applied to evaluate the quality of fish and other meat products, and being a measure of all the volatile nitrogen–containing compounds present in samples via steam distillation. Also, it is one of the most widely used methods today to estimate the degree of decomposition of fish **(Chan *et al.*, 2006)**.

It includes the measurement of *trimethylamine* (TMA) (produced by spoilage bacteria), *dimethylamine* (DMA) (produced by autolytic enzymes during frozen storage), *ammonia* (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile nitrogenous compounds associated with seafood spoilage **(Malle and Poumeyrol, 1989 and Riquixo,1998).** DMA and TMA result of the degradation of trimethylamine oxide (TMAO), a fish typical molecule which has an important role in osmoregulation, DMA is mostly produced by endogenous enzymes and TMA by bacterial enzymes **(Huss**, **1995; Etienne *et al.*, 2005** and **Chebet Lillian, 2007).**

The aim of this work is to describe the quality system based on ISO 17025:2005 and is intended to help and provide laboratory personnel with a consistent, reliable, efficient and professional service with the level of quality required by the laboratory's customers.

Materials and Methods

The system was constructed according to the requirements of the ISO 17025 and Total Volatile Basic Nitrogen (TVB-N) was analysed according to **Commission Regulation (EC) No 2074/2005**.

Technical requirements:

Personnel

The laboratory had staff trained, their competence records and a record of each staff member's formal qualifications, previous experience and date of recruitment also a record of regular re-assessment of the staff member's competence at each of the listed activities. The laboratory manager carried out a competence test on the employee, under direct observation.

A test sample of fish fillet (about one kilo gram) was prepared according to **AOAC (2003)** and analysed under the same conditions (same operator, same apparatus, same chemicals, same laboratory and after short intervals of time) for determination of TVB-N according to **Commission Regulation (EC) No 2074/2005**, **(**10 successive times by each analyst). Repeatability was repeated after one year using canned fish sample.

Accommodation and environmental conditions:

The laboratory followed general rules for good practice in general chemical testing work, provides segregated areas with their own glassware for the storage of standards and the preparation of concentrated solutions and had a system for reporting and recording all spillages. Where foreseeable, have a documented procedure for dealing with specific types of spillage.

Test methods, method validation and quality control:

ISO 17025 requires that the laboratory use appropriate methods which meet the needs of the client and, where possible, methods published as national, regional or international standards. The laboratory quality manual have a statement that the laboratory conforms to the policy of using standard methods and a general policy statement on the laboratory's perception of its own area of expertise. The laboratory use standard method (**Commission Regulation (EC) No 2074/2005)** for determination of total volatile basic nitrogen (TVB-N). In case of usage of non-standard methods, the procedure must be validated **(Garfield 1992).**

Method for determination of total volatile basic nitrogen (TVB-N) describes a reference procedure for identifying the nitrogen concentration of volatile nitrogenous bases (total volatile base- nitrogen) in fish and fish products. TVB-

N is the most commonly used as biochemical method for assessing spoilage. The scope is the extraction of the volatile nitrogenous bases from a sample by strong acid (a solution of 0.6M perchloric acid). After alkalinization the extract is submitted to steam distillation and the volatile bases components are absorbed by an acid receiver. The TVB-N concentration is determined by titration of the absorbed bases. Detection Limits of this procedure is applicable to TVB-N concentration from 5mg/100g to at least 100mg/100g **(Commission Regulation (EC) No 2074/2005).**

Equipment and calibration

Numbering of equipments by the laboratory is very important to avoid confusion.

The laboratory had calibration plan, a brief description and a documented programme for equipment maintenance, calibration and performance verification according to ISO/IEC I7025, as a part of its quality system which has a direct influence on the test results**, (Garfield 1992)**.

The equipments were checked and recorded after each significant repair or modification **(EA 2002).** Defected equipments should be clearly labeled or marked as being out of service until it has been repaired as shown by calibration or tested to perform correctly **(Garfield 1992 and NMKL 1994).**

Weights and balances were calibrated traceably at regular intervals (according to their intended use).

pH meters, were verified regularly and before use. The buffers used for verification purposes were stored in appropriate conditions and were marked with an expiry date.

Kjeltec type distiller was calibrated using reference material (Ammonium sulphate) to detect the percent of nitrogen recovery . Recoveries shall be at least 99.5% **(AOAC, 2003).**

Laboratories were carrying out initial verification for volumetric glassware and then regular checks to ensure that they are performing within the required specification.

The laboratory must guarantee the quality and purity of chemical reagents, standard solutions and purified water used in analysis.

Traceability of measurement

Basically **ISO 17025** requires that a laboratory has in place a calibration system which ensures that, within known limits of uncertainty of measurement. Wherever possible, the international standard is required to be the corresponding SI unit of measurement.

The laboratory had a policy for determining calibration intervals, the object being to ensure that re-calibration takes place before the previous calibration has deteriorated to the point where the validity of the measurements is called into question, **ASCLD/LAB-International (2011) and UKAS (2000).** Using certified reference materials is one way to tackle unknown errors. The laboratory used ammonium sulphate (standard material) to estimate the nitrogen recovery percent **(Fig. 3).**

Maintenance of the quality system:

A good maintenance of the system can be reflected in the results particularly in interlaboratory comparison and proficiency tests, internal quality control which composed of day-to-day and sample-set to sample-set monitoring of analytical performance.

External Quality Control Program (PT):

The laboratory planned to participate in international proficiency tests PT with international proficiency provider organizations to achieve the requirement of accreditation to **ISO 17025:2005**. The test material for proficiency test was dispatched, each participant received a canned fish test material to be analysed for total volatile basic nitrogen (TVB-N).

Internal quality QC control program:

Internal QCs are used to measure accuracy, precision, contamination, and matrix effects, and demonstrate the validity of results. Internal QC schemes utilized for chemistry consist of:

- Control chart (X-Charts): (Rowley, 2009)

A standard material (Ammonium sulfate) was measured 10 times or more. Distill 0.12g ammonium sulfate (preheated at 100C˚ for 1hr.) and titrate against nitrogen with standard Hcl, **(AOAC 2003).**. Approximately 5% of results

were expected to fall outside the 2σ warning limits and any result outside the control limits 3σ requires investigation, also two consecutive results outside the warning limits (UCL and LCL) need an investigation **(Rowley, 2009).**

2.2- Blanks *(negative control):* to determine and measure contamination and interferences. The simplest form of blank is the "reagent blank", where the analytical procedure is executed in all respects apart from the addition of the test portion. It used to assess the preparation batch for possible contamination during the preparation and processing steps, the method blank shall be processed along with and under the same conditions as the associated samples to include all steps of the analytical procedures by using 50,0 ml perchloric acid solution instead of the extract, (blind test), **Commission Regulation (EC) No 2074/2005**. High blank results may indicate contamination either from the solvent, laboratory equipment or laboratory environment and Corrective action must be documented **(Taylor, 1987).**

2.3- Duplicate samples:

The matrix duplicate provides a usable measure of precision which is expressed as relative standard deviation (RSD) or relative percent difference (RPD) of duplicate samples. RPD is calculated when only two sample results are available as follows:

RPD = | R1- R2| /R x 100 where:| R1- R2| = absolute difference between the determinations and R = arithmetic mean of the two values. Duplicate analyses are required. The applied method is correct if the difference between duplicates is not greater than 2 mg/100 g, **Commission Regulation (EC) No 2074/2005**.

2.4- R-Charts

Another graphical display is the R-chart or range chart. When two or more replicate analyses are run on a routine sample or a standard material, the difference between the lowest and highest values in a set of replicates (or just the difference between replicates when there are only two) is called the replicate range (Moving range), **(Figures 1,2,3).** The replicated samples should ideally be within an acceptable total range of concentration, for the same analytical process or methodology **(Delavalle, 1992).** Warning and control limits are calculated as 2.512 times (95 % CI) and 3.267 times (99 % CI) the mean range **(Taylor, 1987).** Since replicate ranges are absolute, only one warning and control limit are displayed **(Figure 1,2)**. Since R-chart data consist solely of replicate ranges, they can only be used to document precision. A minimum of 15 replicated samples is recommended for producing an R-chart **(Taylor, 1987).**

Performance characteristics (uncertainty):

The laboratory was determined the measurement of uncertainty of test results of chemical analyses by the 'Top-down' approach which is based upon Within-lab reproducibility ($\%U_{Rw}$) from the control chart of a standard material or from a control sample completed by data of duplicate analyses on routine samples

$\%U = 2\sqrt{(\%u_{bias}{}^2 + \%u_{Rw}{}^2)}$.

where $\%U_{bias}{}^2$ is the variance of the bias and $\%U_{Rw}{}^2$ is the relative within-lab reproducibility.Accuracy and precision together characterize analytical uncertainty, **FASFC (2008)**.

Reports

The Laboratory was described the data to be included in the formal report which will be sent to the client which include details of the units to be used and any qualifiers to be added to reports, for example uncertainty estimates.

Safety

Any safety precautions to be taken and any hazards known to be associated with the method must be specified. An ISO 17025 assessment does not deal with safety, but the inclusion of safety information in methods is generally regarded as good practice.

Results and Discussion

From the results in **Table (1)** and **Figure (1)** they were shown that the standard deviation of repeatability of four analysts in the first year were 0.64, 0.7, 0.19 and 0.3 and their values of determinations lie between the UCL and the LCL indicating that the four analysts were competent, but the standard deviation result of repeatability of the analyst number five was 1.2 and the TVB-N values lie under the LCL,

also the differences between results were high (high moving range), so he needs more training which led to an improvement in the results of the following year as shown in **Figure (2).** The standard deviation results of repeatability of five analysts in second year were 1.03, 0.84, 0.8, 0.4 and 0.79 and their values of determinations lie between the UCL and the LCL, this means the precision of these determinations is high. The repeatability values (r values) of five analysts were 1.8, 1.97, 0.54, 0.86 and 3.7 in the first year and became 2.9, 2.39, 2.3, 1.14 and 2.23 in the following one respectively. **Commission Regulation (EC) No 2074/2005 reported that the** Standard deviation of comparability SR = 2,50mg/100 g. Standard Deviation (SD), Relative Standard Deviation percent (RSD %) and r value were calculated as shown in **Table (1)** for defining the accuracy and precision **(Currie and Svehla, 1994)**. Competence of staff must be regularly reviewed and normally re-assessed at least annually **(AOAC 2002).** Repeatability determinations are also used to provide confidence in results.

Table (1): Repeatability determinations of Total Volatile Basic Nitrogen (TVB-N):

	Analyst) First year)				
	1	**2**	**3**	**4**	**5**
Mean	30.02	29.91	30.4	30.28	29.36
SD	0.64	0.7	0.19	0.3	1.2
RSD%	2.12	2.33	0.63	1.01	4.1
r value	1.8	1.97	0.54	0.86	3.7
Analyst) Second year)					
Mean	19.72	19.58	19.22	19.77	19.14
SD	1.03	0.84	0.81	0.4	0.79
RSD%	5.2	4.3	4.23	2.05	4.1
r value	2.9	2.39	2.3	1.14	2.23

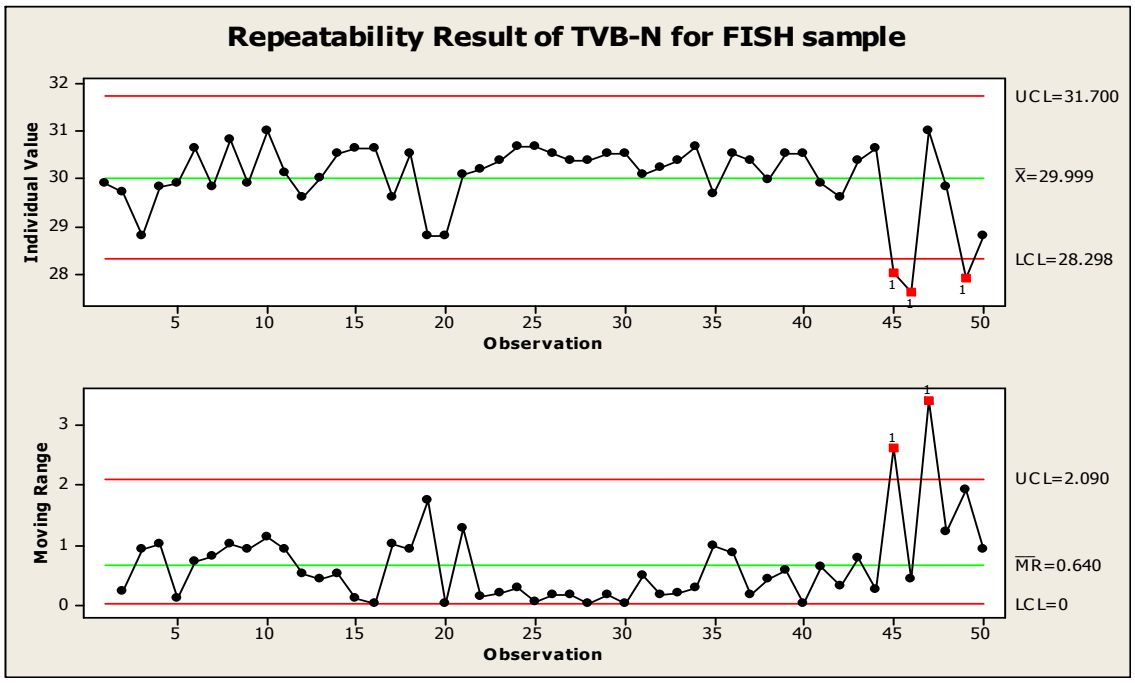
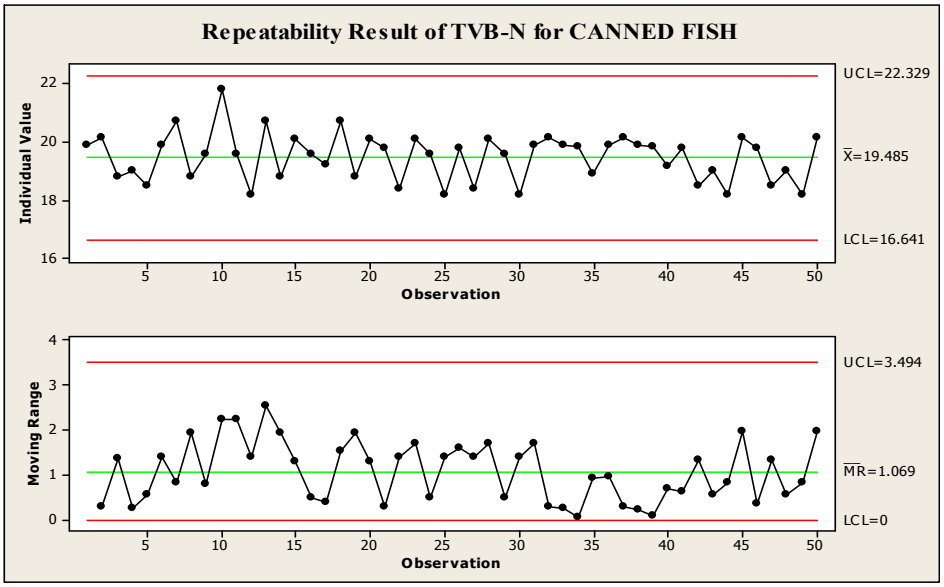


Fig. (1): Repeatability result of TVB-N for Fish sample (first year).



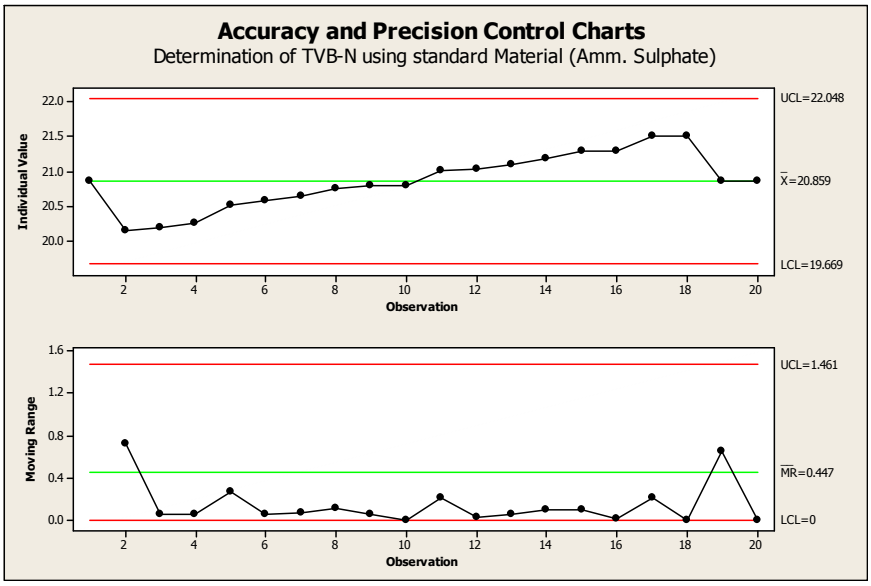
*UCL = upper control limit *LCL= Lower control limit

Fig. (2): Repeatability result of TVB-N for Canned Fish sample (second year).

Proficiency test (PT) results:

In total, 92 sets of test materials were distributed to participants in 39 countries. The assigned value (x_a) (was 37.4 mg/100g of sample) and target standard deviation σ_p were calculated and used to derive a z-score for participants, results. Z-scores are considered satisfactory if $|z| \leq 2$. Result of proficiency test for determination of total volatile basic nitrogen of canned fish was 33.22 mg/100g of sample with $|z| = -1.7$ and the number of satisfactory scores, $|z| \leq 2$ were 63.

Participation in proficiency testing is an important means of quality control and assessing laboratory performance. Article 12 of **EU Regulation (EC) 882/2004** requires laboratories entrusted with official control of food and feed to be assessed and accredited in accordance with **ISO 17025**, so that the proficiency test is an essential element of laboratory quality assurance.



*UCL = Upper Control Limit *LCL = Lower Control Limit

Fig. (3): Control Chart for recovery of nitrogen using standard material (Amm. sulphate)

Figure (3) shows the average value and the standard deviation (SD). In a normally distributed sample population, +/- 2 SD represents a 95 % confidence interval (CI) and +/- 3 SD corresponds to a 99 % CI. An individual value between UWL and UCL or LWL and LCL is considered acceptable. **(Delavalle, 1992 and SSSA, 2004)** The laboratory calibrated its method periodically and checked its performance by testing the standard material and so established traceability. X-charts are especially useful as a day to day tool to monitor for ongoing or emerging problems. The Laboratory used accuracy and precision control charts to determine if the measurement system process is in control and whether the results generated by the measurement system are acceptable.

Conclusion

From the present study, it was concluded that accreditation cannot be reached in one moment, but gradually with improvement of organization, records, equipment, methods and personnel training all organized in a quality manual always in improvement.

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تطبيق نظم الجودة للمواصفه أيزو 17025 : 2005 لأختبار تقدير نسبة النيتروجين الكلى المتصاعد فى الأسماك

أميمة محمد الطاهر لبيب المغربي – إيمان المسلمى - مصطفي محمد محمد حسوبة – ليلى على أحمد
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الملخص العربى
قامت الدراسة الراهنة بعمل وثيقة لتحديد المبادىء التوجيهية لتنفيذ مراقبة الجودة الداخلية للمختبرات (IQC) التحليلية للمتطلبات العامة للكفاءة فى المواصفه أيزو 17025:2005 وذلك لتحديد نسبه النيتروجين الكلى المتصاعد للأسماك ومنتجاتها, وتم اجراء الأستعراض الأحصائى لنتائج تكرار الاختبار للأطباء الفاحصين لمرات عديدة فى فترات زمنية متقاربة, التى اثبتت قدرتهم على اجراء الاختبار بدقه وكفاءة عالية, وكانت نتائج اختبارات الكفاءة الدولية مع المنظمات الدولية ناجحه, حيث كانت نسبة النيتروجين الكلى المتصاعد لعينة الأسماك المعلبة 33,3 ملجم/100جم من العينة وقيمتها المقدرة من المعمل الدولى 37,4 ملجم/ 100 جم من العينة, وكان Z score أقل من 2 (-1.7) مما أدى إلى حصول المعمل على شهادة فى مجال الأختبار والمعايرة من المجلس الوطنى للاعتماد لأختبار تقدير نسبة النيتروجين الكلى المتصاعد للأسماك ومنتجاتها.