Accreditation according to ISO/IEC 17025 : 2005 of the compositional analysis in food chemistry laboratories

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Abstract

This document sets out guidelines for the implementation of internal quality control (IQC) procedures in analytical laboratories based specifically on ISO standard 17025 for determination of Protein (as Nitrogen), Ash, Moisture and Fat%. The standard deviation results for determination of protein, fat, ash and moisture% of all analysts ranged from 0.001 to 2.3 in the first year and from 0.01 to 0.92 in the second year, and most of the determination values lie between the upper control limit (UCL) and the lower control limit (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 protein (as nitrogen %), total fat, ash and moisture% to achieve the requirement of accreditation to ISO 17025:2005. The assigned values (x_a) (were 2.45, 8.96, 2.85 and 70.57 g/100g of sample, respectively) and the laboratory results were 2.44, 10.15, 2.84 and 70.71 g/100g of sample respectively with $|z| = -0.3, 1.9, -0.1$ and 0.2 respectively. Z-scores are considered satisfactory if $|z| \leq 2$. 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, Protein, Ash, Moisture and Fat .*

Introduction

Implementing an ISO/IEC 17025 laboratory management system is a means to ensure efficiency and technical competency in testing laboratories. A laboratory that establishes a laboratory management system compliant with ISO/IEC 17025 joins the growing world partnership of accredited laboratories, and will gain international recognition for its commitment to quality, competency and reliable results. In addition, ISO/IEC 17025 accreditation will signify that the laboratory comply with an internationally recognized standards, thus easing the global exchange of valuable information. ISO/IEC 17025 is an ideal management system model for laboratories because it aims to control quality costs, improve measurement accuracy and guarantee consistency of results. It is also customer-driven, when implemented correctly, the elements of ISO/IEC 17025 work meticulously together to ensure that required quality levels are met and that customers' needs are satisfied.

Quality has become an important issue to people all over the world. ISO/IEC 17025 accreditation provides the assurance that testing laboratories are delivering good services, and consistent data. **The Egyptian Accreditation Council, 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 recognised in most countries of the world. 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 certifi-

cation of products & systems as well as personnel. **Jose Fu Wong (2000)** analyzed the sections and clauses that are to be included in a laboratory's quality manual based on the European Standard EN 45001:1989 "General criteria for the operation of testing laboratories" in order to comply with the requirements of the new ISO Standard. A "diagnosis audit" to this quality manual was performed using the applicable requirements of ISO/IEC 17025. The content of these two standards was compared for supplementing and drafting the missing sections. The main conclusions from the audit point out those seven clauses from ISO/IEC 17025 need to be included in the quality manual. **Wiegers (2003)** reported that the third-party accreditation is a valuable tool to demonstrate a laboratory's competence to conduct testing and accreditation is only I part of establishing data credibility. **Kawai (2004)** reported that the International Organization for Standardization (ISO) have published two international standards (IS) to be used for accreditation of clinical laboratories; ISO/IEC 17025:1999 and ISO 15189:2003. Any laboratory accreditation body must satisfy the requirements stated in ISO/ IEC Guide 58. In order to maintain the quality of the laboratory accreditation bodies worldwide, the International Laboratory Accreditation Cooperation (ILAC) has established the mutual recognition arrangement (MRA). **Drolc *et al*. (2005)** recognised the Measurement of traceability as one of the basic prerequisites for comparability of results obtained in different laboratories and is a basic aspect of metrological sciences such as analytical chemistry. This requirement is underscored by the increasing adoption of standards and measurement quality systems, such as laboratory accreditation against ISO/IEC 17025. Testing laboratories ensure traceability of their measurement results by using appropriate reference standards for calibration of instruments and control of measurement processes. **Rowley (2009)** and **Tracy Szerszen (2009)** wrote a guidebook to assist testing and calibration laboratories set up a quality management system that conforms to ISO 17025:2005, the international standard for laboratory quality systems. Compliance with this standard provides a globally accepted basis for laboratory accreditation. The standard specifies the management and technical requirements to be met by testing and calibration laboratories in both their organization and their management of quality. Complying with ISO 17025 will enable laboratories to identify where their current operations meet the requirements of ISO 17025 and, in those areas where they do not, it will guide them in developing systems to achieve such compliance.

The food analysis laboratories were mainly concerned with compositional analysis of food and food products to assess according to legal requirements of Egyptian Standards. Early food composition studies were carried out to identify and determine the chemical nature of principles in foods that affect human health (protein, fat, and carbohydrate). Nationally and internationally, compositional data are used in the assessment of the nutritional value of the food consumed by individuals and populations. Also establishing the composition of foods often has advantages for the trade in foods because importing countries with nutrition-labeling regulations prefer (and may require) that imported foods conform to the standards expected of locally produced foods.

Materials and Methods

The system was constructed according to the requirements of the ISO 17025: 2005; Protein, Ash and Moisture% were analysed according to **AOAC, 2003**, and Fat% was analysed according to **ISO 1443/1973** and **Commission Directive 98/64/EC (1998).**

Technical requirements:

a- 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 as a main requirement of **ISO 17025 (2005)**

and FAO **(2011)**.

Different samples of luncheon, dried milk and meat (about one kilo gram of each) were 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 Protein, Fat, Ash and Moisture% **(**10 successive times by each analyst). Repeatability was repeated after one year using canned meat, dried and raw milk samples.

b- 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.

c- Test methods, method validation and quality control:

ISO 17025 requires that the laboratory uses 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 uses standard method **AOAC (2003)** for determination of protein, ash and moisture%, and **ISO 1443/(1973) and Commission Directive 98/64/EC (1998)** for determination of fat%. In case of usage of non-standard methods, the procedure must be validated **(Garfield, 1992).** Compositional analysis refers to the determination of the major constituents of food, and is used to assess if the food is within normal compositional parameters, or has somehow been adulterated. The four usual constituents measured for compositional analysis are protein, fat, ash and moisture.

d- 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 equipments maintenance, calibration and performance verification according to **ISO/IEC I7025 (2005)**, 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 equipment 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).** Calibration programmes established depending on the specific requirements of the analysis **(UKAS 2000).**

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

Hot air oven and Muffle furnace were calibrated traceably at regular intervals and checked every use with calibrated thermometer.

Kjeltec type distiller and Digestion unit were calibrated using reference material (Certified Reference Tryptophan and Ammonium sulphate) to detect the percent of nitrogen recovery. Recoveries shall be at least 98% and 99% respectively, **(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, standards solutions and purified water used in analysis.

e- 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 Tryptophan (Certified Reference Material) and Ammonium sulphate (standard material) to estimate the nitrogen recovery percent **(Fig. 9).**

f- 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.

1- 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 meat test material to be analysed for protein (nitrogen %), total fat, ash and moisture%.

2- 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) for determination of nitrogen recovery: (Rowley, 2009)

A standard material (Ammonium sulfate) was measured 10 times or more. 0.12g ammonium sulfate (preheated at 100C° for 1hr.) and 0.85g sucrose per flask. Adding all reagents as stated in test portion preparation were digested and distilled under same conditions. Titrate against nitrogen with standard HCl, **AOAC (2003).**

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 is 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 (blind test). Two empty dishes were blank for moisture determination; also empty incineration dish and ashless filter paper instead of sample were blank for ash determination. 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.

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). 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 **(Figures 1- 8).** 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).**

2.5. Quality Control sample:

In each batch a control standard (QC sample) should be analysed. The laboratory QC sample can be made from dry milk powder or animal

feed. Take 1 kg of the chosen QC sample, and stored in a cool, dry place. Analyse the QC sample 15–20 times, take average and allow ± 2 SD as an acceptable range. Samples should be analysed in duplicate. For animal feed, the difference between the values of two parallel determinations carried out on the same sample shall not exceed 0.2% in the absolute terms for contents lower than 10%, and 2% relative to the higher value for contents ≥ 10% (with a maximum difference of 0.5%-units) **(FAO, 2011).**

g- Performance characteristics (uncertainnty):

The laboratory has determined the measurement of uncertainty of test results of protein 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

$\%UR_w^2 = RSD_{CC}^2 + RSD_r^2$

Where $\%UR_w$ the relative measurement uncertainty on the within-lab reproducibility, RSD_{CC} is the relative standard deviation as deduced from the control chart and RSD_r is the relative standard deviation under repeatability conditions. Estimating the expanded measurement uncertainty U for calculating the expanded measurement uncertainty %U for determination of fat, ash and moisture%.

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

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).**

h- Reports

The Laboratory has 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 **Figures (1 to 8)** showed that the standard deviation results of repeatability for determination of protein, fat, ash and moisture% of all analysts ranged from 0.001 to 2.3 in the first year and from 0.01 to 0.92 in the second year, and most of the determination values lie between the UCL and the LCL indicating that the analysts were competent. So training leads to an improvement in the results of repeatability on the following year as shown in **Figure (2, 4, 6 and 8)** compared to **Figures (1, 3, 5 and 7),** this means the precision of these determinations is high. The repeatability values (r values) ranged from 0.004 to 6.5 in the first year and became ranged from 0.03 to 2.6 in the following one respectively. Standard Deviation (SD), Relative Standard Deviation percent (RSD %) and r value were calculated as shown in **Table (1)** to define 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. **AOAC (2003)** reported that the repeatability values (r) established in the collaborative study for determination of water% of milk, protein % of mozzarella cheese and nitrogen% in milk were 0.052, 0.622 and 0.083, and RSD% were 0.149, 0.82 and 0.385 respectively.

Table (1*).* Repeatability for determination of Protein, Fat, Ash and Moisture (Water) %.

	Analysts	First year				Second year			
		Mean	SD	RSD%	r value	Mean	SD	RSD%	r value
Protein%	1	1.22	0.085	6.98	0.24	2.4	0.069	2.85	0.19
	2	1.19	0.083	6.93	0.23	2.43	0.053	2.16	0.15
	3	1.17	0.034	2.9	0.097	2.44	0.045	1.86	0.13
	4	1.15	0.001	0.12	0.004	2.41	0.056	2.32	0.16
	5	-	-	-	-	-	-	-	-
Fat%	1	-	-	-	-	-	-	-	-
	2	16.8	1.9	11.2	5.3	10.6	0.57	5.4	1.6
	3	15.7	2.3	14.6	6.5	10.2	0.68	6.7	1.9
	4	15.7	1.01	6.4	2.85	10.2	0.65	6.4	1.9
	5	15.5	1.53	9.9	4.3	10.4	0.92	8.85	2.6
Ash%	1	0.99	0.02	1.55	0.04	4.5	0.12	2.7	0.35
	2	0.94	0.05	5.8	0.15	4.6	0.13	2.8	0.36
	3	0.99	0.01	1.3	0.04	4.5	0.03	0.7	0.08
	4	0.93	0.03	3.6	0.1	4.6	0.01	0.2	0.03
	5	0.94	0.05	5.1	0.14	4.46	0.13	2.8	0.36
Mois-ture%	1	65.8	0.88	1.33	2.48	84.6	0.39	0.46	1.1
	2	65.4	0.82	1.26	2.3	84.5	0.07	0.085	0.2
	3	65.4	0.29	0.44	0.82	84.5	0.3	0.38	0.92
	4	65.6	0.23	0.35	0.66	84.6	0.2	0.23	0.56
	5	64.3	0.95	1.5	2.7	84.7	0.29	0.34	0.82

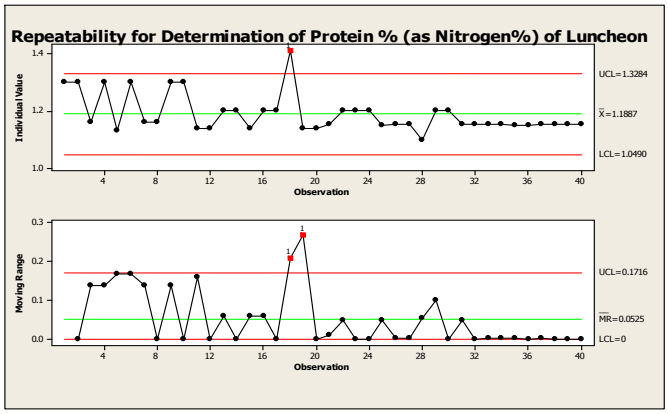
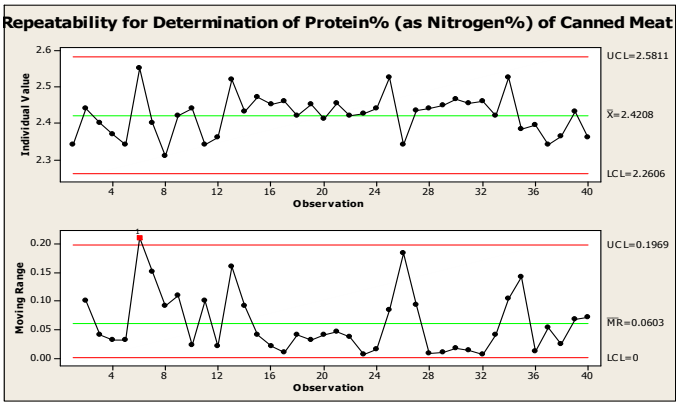


Fig.(1*).* Repeatability for Determination of Protein % (as Nitrogen%) of Luncheon (First year).



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

Fig. (2*).* Repeatability for Determination of Protein% (as Nitrogen%) of Canned Meat (second year).

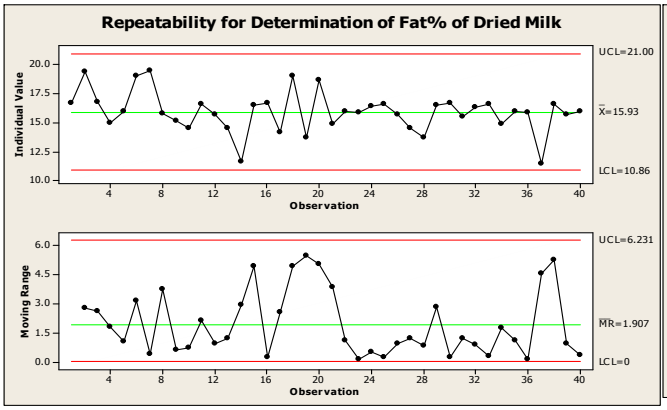
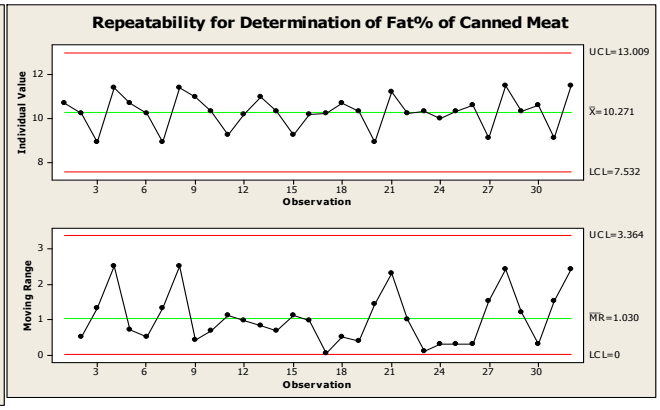


Fig. (3). Repeatability for Determination of Fat% of Dried Milk. (First year).



UCL = upper control limit
*LCL= Lower control limit

Fig. (4). Repeatability for Determination of Fat% of Canned Meat (second year)

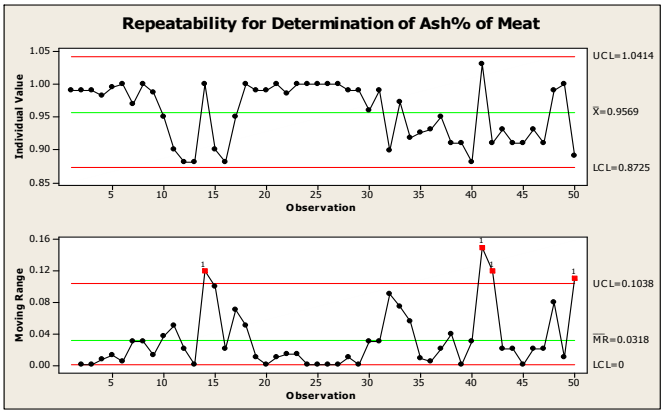
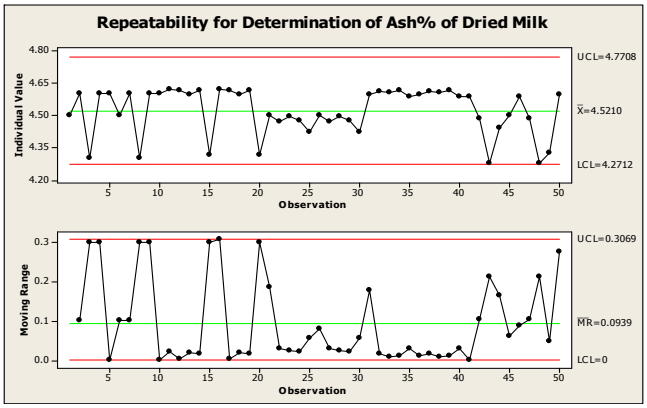


Fig. (5). Repeatability for Determination of Ash% of Meat. (first year)



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

Fig. (6). Repeatability for Determination of Ash% of Dried Milk. (second year)

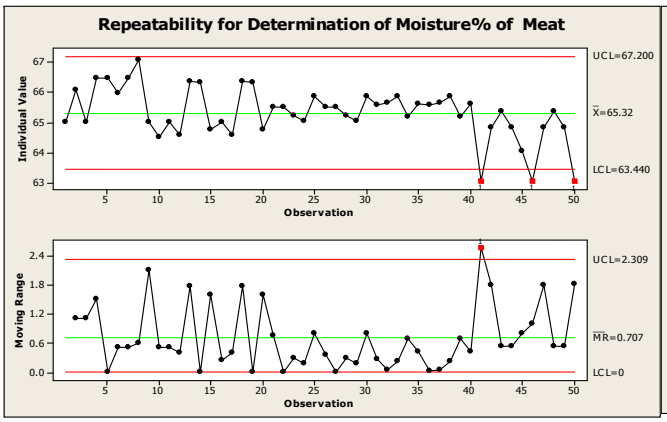
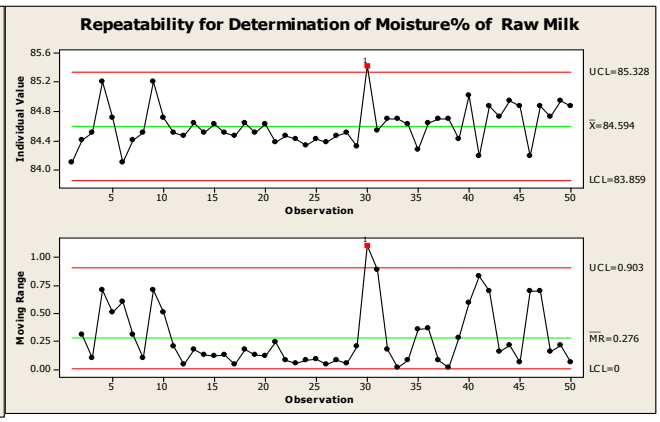


Fig. (7). Repeatability for Determination of Moisture% of Meat. (first year)



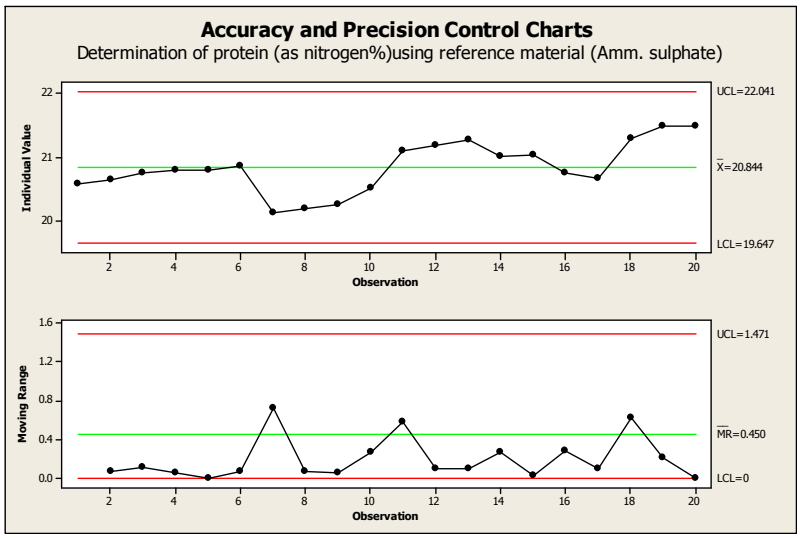
UCL = upper control limit
*LCL= Lower control limit

Fig. (8). Repeatability for Determination of Water % of Raw Milk. (Second year)

Proficiency test (PT) results:

In total, 112 sets of test materials were distributed to participants in 36 countries. The assigned value (x_a) (were 2.45, 8.96, 2.85 and 70.57 g/100g of sample, respectively) 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 protein (nitrogen %), total fat, ash and moisture% of canned meat were 2.44, 10.15, 2.84 and 70.71 g/100g of sample respectively with $|z|$ = -0.3, 1.9, -0.1 and 0.2 respectively. The numbers of satisfactory scores, $|z| \leq 2$ were 80, 89, 91 and 103 respectively. 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:(2005)**, so that the proficiency test is an essential element of laboratory quality assurance.

Figure (9) illustrated Control chart (X-Charts) for determination of nitrogen recovery which gives a general visual indication when any systematic drift in the values returned by standard material is setting in. A method which is being used under control should show a random distribution of the actual values about the expected result, and any trends developing which suggest a bias should be investigated. 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).** This Figure shows the average value and the standard deviation (Sd). In a normally distributed sample population, +/- 2 SD represents a 95 % confidence interval (CI) (warning limits, WL) and +/- 3 SD corresponds to a 99 % CI (Control limits, CL). An individual value between UWL (upper warning limit) and UCL or LWL (lower warning limit) and LCL is considered acceptable. **(Delavalle, 1992** and **SSSA, 2004).** The laboratory calibrated its method periodically and checks its performance by testing the standard material and so establishes 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.



*UCL = upper control limit

*LCL= Lower control limit

Fig. (9*).* Control Chart for recovery of nitrogen using standard material (Amm. sulphate)

Conclusion

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

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تطبيق نظم الجودة للمواصفه أيزو 17025 : 2005 للأختبارات التحليلية فى معامل كيمياء الأغذية

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معهد بحوث صحة الحيوان – قسم صحة الاغذية – دقي – جيزة

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 البروتين (من خلال تقدير نسبة النيتروجين), الدهون, الرماد والرطوبة 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, الدهون الكلية، الرماد والرطوبة لعينة اللحوم المعلبة 2،44؛ 10،15؛ 2،84 و 70،71 على التوالى بينما كانت نتائج المعمل الدولى 2،45؛ 8،96؛ 2،85 و70،57 على التوالى. وكان Z scoreأقل من 2 (- 0,3؛ 1،9؛ -0،1 و 0,2) مما أدى إلى حصول المعمل على شهادة فى مجال الأختبار والمعايرة من المجلس الوطنى للاعتماد لأختبار تقدير نسبة النيتروجين , الدهون الكلية, الرماد والرطوبة فى الأغذية والأعلاف.