الشركة القابضة
لمياه الشرب والصرف الصحى

برنامج المسار الوظيفي
للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

QA/QC

كيميائي مياه- الدرجة الثالثة

تم اعداد المادة بواسطة الشركة القابضة لمياه الشرب والصرف الصحي.
الادارة العامة لتخطيط المسار الوظيفي.الإصدار الثانى –2019 .

مقدمة الإصدار الثانى

تهدف مجموعة البرامج التدريبية المعدة من إدارة المسار الوظيفى بالشركة القابضة لمياه الشرب والصرف الصحى إلى رفع كفاءة الكيميائين العاملين بالشركة القابضة لمياه الشرب والصرف الصحى والشركات التابعة لها وتنمية مهاراتهم ومعارفهم بالشكل الذى يضمن الوصول إلى كوب مياه نظيف وبيئة آمنة يرضى متطلبات وإحتياجات العملاء الكرام.

ويعتبر الإصدار الثانى من برامج المسار الوظيفى لوظيفة كيميائى مياه الشرب هو ثمرة جهود الكيميائيين العاملين بمعامل الشركات التابعة والمعمل المرجعى لمياه الشرب بالشركة القابضة بما تحمله من مزيج متجانس من الخبرات والكفاءات الذين لم يدخروا جهدا حتى يخرج هذا العمل بالطريقة اللائقة.

وجدير بالذكر أن هذا الإصدار يعتبر مكتبة مرجعية وافية وشاملة لجميع الجدارات المتضمنة المهارات والمعارف التي تجعل الكيميائى كفؤا لوظيفته.

ومما تجدر الإشارة إليه بأنه تم الاعتماد على منهجية للمسار التدريبى بحيث يكون المتدرب قد تلقى الدورات الحقلية بداية من التعرف على مراحل التنقية والمعالجة ثم الانتقال إلى الدورات المعملية داخل معمله طبقا للإطار الزمنى المحدد للمدد البينية لكل درجة وظيفية.

ولقد اعتمدنا على وضع معايير لكل مرحلة فى إعداد هذا الاصدار وكان من أهم هذه المعايير:

- المشاركة الفعالة للخبرات والكفاءات التدريبية بالشركات التابعة فى وضع المناهج بما يناسب عموم الكيميائيين على مستوى الجمهورية.
- عقد ورشة عمل متخصصة لكل مادة تدريبية يشارك بها جميع المدربين ذوى التخصص والخبرات سواء من المعمل المرجعى أو معامل الشركات فضلا عن أن يكون المدرب قد قام بتدريس هذه المادة مرات عديدة.
- استخدام وسيلة اتصال غير تزامنى بين جميع المدربين المعتمدين لكل مادة على حدة من خلال انشاء جروب على الفيس بوك لكل مادة على حده (مذكور فى دليل المدرب).
- وضع حقيبة تدريبية كاملة لكل برنامج معدة طبقا لأحدث النظم والمعايير العالمية تحتوى على (دليل المتدرب- شرائح العرض- ملحقات مقروءة ومرئية- دليل المدرب- بنك الأسئلة).
- بناء المحتوى لكل برنامج تدريبى طبقاً لأحدث المراجع العالمية ومن أهمها كتاب الطرق القياسية لتحليل مياه الشرب والصرف الصحى (الإصدار رقم 23) وبما يتوافق مع متطلبات آخر إصدارات الايزو(17025)، مع مراعاة التحديثات الخاصة بالتشريعات والقوانين ذات الصلة.

وجدير بالذكر أن الإصدار الثانى من البرامج التدريبية اعتمد فى تصميمه على عرض مبسط للمعلومات قدر الامكان طبقاً للأسس العلمية وطبقاً للجدارات المعتمدة على تحديد أهداف واضحة وصريحة لتدريب المتدربين، وتشتق تلك الجدارات من الفهم الواضح لدور المتدرب طبقا لبطاقة الوصف الوظيفى، وتتضمن معارف ومهارات وسلوك. مما يضمن إكساب المتدرب مهارات سلوكية بالإضافة إلى المواد التخصصية.

كما تم تصميم العديد من ورش العمل على أساس تسهيل و تسريع عمليتي التعلم و كسب المهارات بما يسمح بتعظيم الفائدة من العملية التدريبية.

كذلك تم استخدام أساليب التدريب الحديثة والاعتماد على التدريب التفاعلى والتركيز على الجوانب التطبيقية في استخدام الوسائل والأساليب المختلفة ، كما تم استخدام الطرق الحديثة للتعليم التفاعلي والغير تزامني كمصادر مساندة للتعلم من خلال انشاء جروب على الفيس بوك للمدربين المعتمدين (**HCWW Trainers**) .

وفى الختام نرجوا من الله أن يتقبل منا هذا العمل كما نأمل أن يكون هذا العمل علما نافعا للعاملين بقطاع المعامل بالشركة القابضة والشركات التابعة لما يشمله من معلومات فنية قيمة وأن يفيد العاملين الجدد بها ليصبحوا قادرين على تنفيذ مهامهم الوظيفية بالشكل الأمثل .

والله ولي التوفيق.

I. Introduction

Industrial QC was introduced in the 1930s by **Shewhart**, a statistician at Bell Laboratories. He published a text provided the theory and practice guidelines for statistical quality control. The technique recommended was to sample a group of products and determine the mean and range of critical characteristics, this technique is still standard practice in industry today.

In the 1940s, **Deming** (who worked with Shewhart at one time) was charged with providing training in SQC to American industry to assure the quality of wartime production. One of the strengths of American armaments was the quality of production and SQC became widely practiced in American industry.

In the late 40s and 1950s, Deming was asked to assist Japanese industry in improving the quality of production, particularly telephones, which were needed to improve communications. In addition, **Juran** began to provide broader training in quality management. Their efforts led to the principles and practices of Total Quality Management (TQM) in industry.

In water sector, production of high quality drinking water demands adequate quality monitoring program for the water from intake to the tap. Hence, the laboratory has played an important role in drinking water production by providing the management with the required information. This information has to be technically valid, legally defensible, and of known quality.

For this regard, the laboratories strive to establish and implement quality systems to guarantee the reliability and traceability of the generated information. Establishing of quality system is costly and time consuming, however, these milestones is relatively low in comparison to the advantages gained.

Generally, the main advantage of implementing quality systems is: (i) the positive impact on the organization's image in the market and society; (ii) the documentation system which is the laboratory's memory and a helpful tool that facilities the communication among the staff for articulating common principles. In the central laboratories consisting of many laboratories, each operating complex procedures, the more likely it is that misunderstandings and mistakes will have occurred and been adopted even though they are bad practice.

Even in small laboratories, the absence of a member of staff who is on holiday or ill can cause confusion. If operating procedures are written down for staff to refer to, as part of the quality management system, the number of such mistakes will be reduced

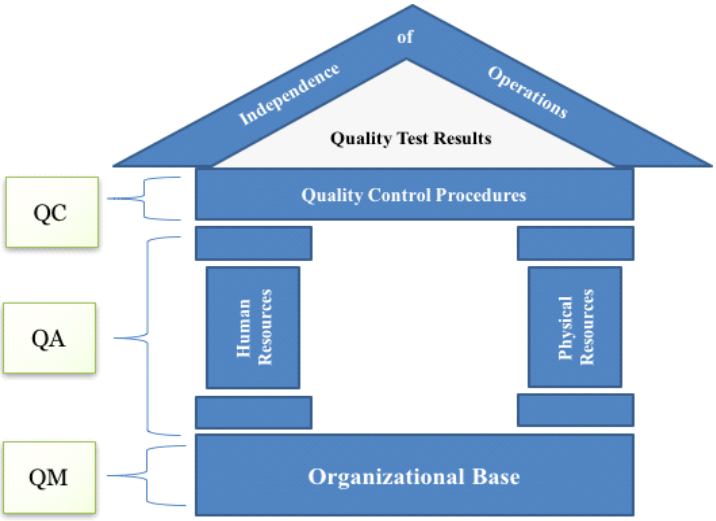
II. Principals

2.1. Quality

- **According to ISO**
  - Quality is the totality of features and characteristics of a product or service that bears on its ability to satisfy given needs.
- **In analytical methods**
  - Quality is "Delivery of reliable information within an agreed span of time under agreed conditions (Precision, Accuracy), according to agreed costs"

2.2. Quality Management System

- It is a management system to direct and control an organization with regard to quality.



2.3. Quality Management (QM)

- Coordinated activities to direct and control an organization with regard to quality

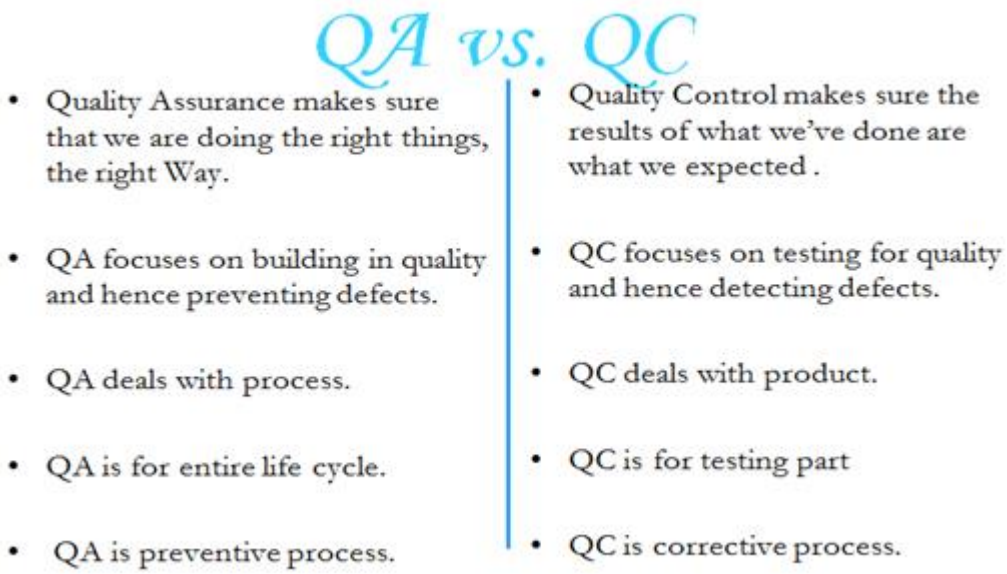
2.4. Quality Assurance (QA)

- A definitive plan for laboratory operations that specifies the measures used to produce data with known precision and bias.

2.5. Quality Control (QC)

- Set of measures used during an analytical method to ensure that the process is within specified control parameters.

2.6. Difference between QA/QC



2.7. Quality System Structure

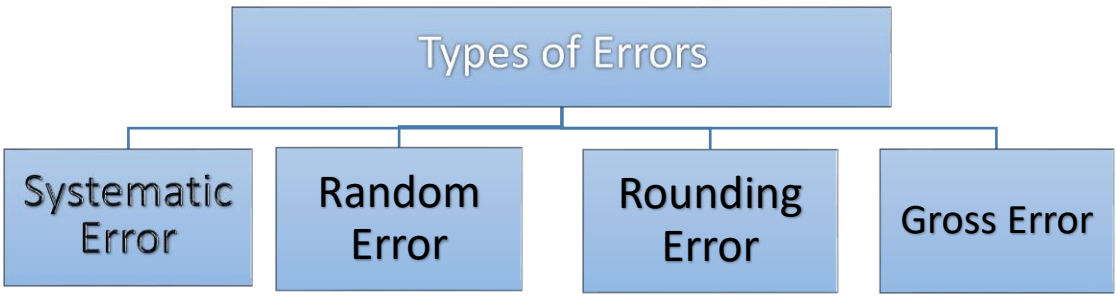
2.8 Accuracy

- Accuracy is the closeness of a measurement or the mean of a set of measurements to the true value. Accuracy is assessed in terms of percent recovery for quality control check samples and matrix spikes.

2.8. Precision

- Precision is the closeness between a set of replicate measurements without assumption or knowledge of the true value. Analytical precision is assessed by means of laboratory duplicate or replicate.

2.9. Errors:



2.9.1. Causes of Systematic Error

- Instability between sampling and measurement
- Inability to determine all relevant forms of the analyte
- Interference
- Biased calibration
- Biased blank

2.9.2. Causes of Random Error

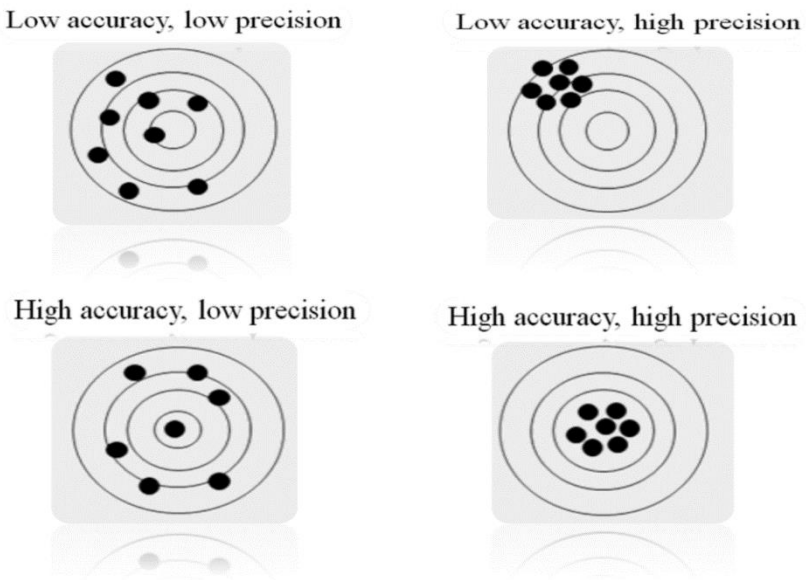
- Uncontrolled variations in the conditions of the measurement system during different measurements, e.g.:
  - Differences in volume
  - Fluctuations in temperature or electrical current.
  - Reading of scales
  - Differences in calibration between batches

2.9.3. Causes of Gross Error:

- Personnel mistakes or carelessness:
  - Using wrong method.
  - Misreading a scale or signal display.
  - Analysis of wrong sample.
  - Erroneous marking of sample.
  - Results in wrong unit.
  - Calculation error.
  - Transposition of data.
  - Transcription error.

2.9.4.Causes of Rounding Error:

- Either rounding off data so much that the natural variability is obscured or keeping so many of the digits produced by a calculator or an instrument that a false impression of accuracy is produced



III. Quality System

- We have to define its elements clearly and correctly.
- The main elements of the quality system are QM, QA & QC as mentioned above. In this section, we will discuss in details QA (Human and Physical Resources) & QC practices whilst QM will be discussed at the next level.

3.1. Personnel

3.1.1. Qualification:

- The laboratory shall have a complete information about all the staff includingcertification, training programs, experiences, and Job descriptions. (***Appendix 01*** *- list of laboratory staff,* ***Appendix 02*** *- CV*).

3.1.2. Training:

- Training requirements shall be outlined and documented on the basis of the position duties description (***Appendix 05*** *- training needs*, ***Appendix 06*** *- training plan*)
- The level of training is determined by the staff qualifications, experience, complexity of the test method, and knowledge of the test method performed.
- The staff should not perform any procedure, or method until all applicable on job training has been completed and competency demonstrated (***Appendix 07*** *- on job training*)
- Personnel undergoing training are supervised until training is completed and competency demonstrated (***Appendix 08*** *– evaluation after training*).

3.1.3. Competency:

- The performance of the laboratory staff shall be demonstrated according to their experiences, qualifications. The annual performance check plan is mentioned in ***Appendix 09.***
- Some techniques are consulted to assess the performance of the technical staff including some statistical tools.
- ***NOTE:*** Check performance tools may include but not limited to (i) achieving an objective (ii) Observation of procedure or process (iii) Oral query (iv) Conducting training (v) Evaluation of precision in both terms of repeatability limit (r) and reproducibility limit (R) where the difference between two readings x_1 and x_2 should be less than repeatability limit (r) for a person and less than reproducibility limit (R) for a working group (vi) Evaluation of

accuracy based on Recovery as mentioned below in quality control section. The results of this check have to be recorded including the recommendations of the inspector ***(Appendix 10 –*** *check performance record****)***

3.1.4.Authorization:

- the authorization should be completed for each one and kept in the personnel file (Appendix 03 – authorization)
- Lab. shall maintain relevant delegations for key positions, original position occupier and the delegated person by names. These delegations should be signed, approved, and displayed; also it should include description tasks (Appendix 04 - delegation)

3.2. Facilities, Safety and environmental conditions

3.2.1.Facility

- Laboratory's installation shall facilitate correct performance of the tests and also the technical requirements for accommodation and environmental conditions affect results shall be documented ***(Appendix 11 –*** *environmental record****)***

3.2.2.Safety

- All the safety requirements should be established and demonstrated as follows:
  - Occupational health and safety guide should be obeyed in both terms of individuals and working area (Annex I).
  - Distinguish high risk chemicals and retain MSDS for all laboratory chemicals.
  - Emergency plan (***Appendix 12***) should be available and should include the following:
    - Spill / volatilization of chemicals
    - Fire
    - Explosion
    - Warning signs should be taken into account
  - Occupational health and safety equipment should be available (withdrawal gas cabinets, ventilation hoods, shower, washing eyes and fireextinguishers).
  - Laboratory staff should be trained to deal with emergency situations

3.2.3.Environmental Condition

- Effective separation between neighboring areas should be present to prevent cross contamination.

3.2.4.Housekeeping

- Eating, drinking and smoking are prohibited at the laboratory.
- The lab. shall control access to the laboratory and use of area effect on quality of tests (***Appendix 13*** - *Staff Only*)
- Laboratories areas are maintained sufficiently clean and orderly to prevent contamination of tests and to facilitate the efficiency of laboratory operations. The laboratory shall have a regular cleaning and disinfection plan that carried out in order to keep the premises in a condition suitable for conducting tests (A***ppendix 14*** *– daily housekeeping*, ***Appendix 15*** *– weekly and monthly housekeeping*)

3.3. Equipment & Chemicals

3.3.1.Equipment

- The lab shall be equipped with the required equipment and tools that achieve the accuracy stated by the methods. The following items are required:
  - A list of instruments and glassware to be verified should be available ***(Appendix 16*** *– equipment list*, ***Appendix 17*** *– glassware list*)
  - The lab shall have a plan for equipment calibration (***Appendix 18*** *– Calibration plan*).
  - Verification should be done by following the manufacturer's instructions, equipment guides and the methods. (As mentioned in Annex II)
  - Records for each laboratory instrument should be available (Appendix 19 – equipment record sheet)
  - Minimum records are:
    - ✓ Date of installation and date of first operation
    - ✓ Short cuts of the manufacturer operation instructions
    - ✓ Monitoring record (if appropriate).
    - ✓ Calibration certificates.
    - ✓ Maintenance record.
    - ✓ Identification card (not label).
    - ✓ Cleaning record (if appropriate).

- ✓ Working instruction or manufacturer manual.

➢ The lab shall periodically plan for the activities including intermediate calibration checks (***Appendix 20*** *–intermediate calibration checks plan*) and maintenance (***Appendix 21*** *–maintenance plan*)

➢ Log book entries should be signed.

➢ Identification labels should be attached to all instruments/equipment.

➢ The identification labels for instruments/equipment should show as minimum:

- ✓ ID
- ✓ Last and next calibration date
- ✓ Serial number

3.3.2.Chemicals

- A list of purchased, prepared chemicals and standard materials should be available including MSDS certificate and certificate of analysis as essential requirements certificate of analysis for reference materials , Uncertainty value, and manufacturer should be complying to ISO 17034(***Appendix 22*** *– purchased chemicals,* ***Appendix 23*** *– prepared chemicals,* ***Appendix 24*** *-standard materials*)
- Identification labels should be attached to all chemicals/solutions mentioning the following:

  For prepared chemicals:

  ➢ Name of contents, Formula, Concentration, Date of manufacture / Preparation and Expiry date.

  For purchased chemicals: unique ID and date of opening are enough.
- Hazardous chemicals and solutions should be marked with relevant hazard symbols.
- A system followed for correct storage of chemicals and glassware should be available in the laboratory. The system should include an updated inventory book(s) for chemicals and glassware (***Appendix 25*** *– consumed chemicals,* ***Appendix 26*** *– consumed glassware*)
- The inventory for chemicals should include names, expiry dates, updated stock and signature.
- The inventory for glassware should include names, updated stock and signature.

- Storage of chemicals should be done according to the manufacturer recommendations.
- The system should include arrangements for safe disposal of expired chemicals.

3.4. Methods

- The laboratory shall use appropriate methods and procedures for all laboratory activities considering its resources **(*Appendix 27 – list of laboratory methods*).**

3.4.1. Types of Methods

- Standard Methods.
- Official Methods [for use by government organization (EPA, NIOSH)].
- Literature Methods [Analytical Journals].
- In-House Developed Methods [Laboratory procedures].

3.4.2. Method Validation and verification

- **Validation:** is the procedure used to prove that a test method consistently yields what it is expected and required to do with adequate accuracy and precision.
- **Verification:** is the process to demonstrate the competence in test performance of an already validated standardized test method.

3.4.3. Method should be validated when:

- Changes in established methods
- New method developed for a particular problem.

3.4.4. Method Validation Parameters:

- Calibration function/linearity.
- Sensitivity.
- Limit of detection.
- Limit of Quantitation.
- Precision.
- Accuracy.
- Range of application.
- Selectivity/Interferences
- Ruggedness / Robustness

3.4.5. Method should be Verified when:

- Characteristics of Standard Method Should be verified before initiation of method in laboratory.

3.4.6. Verification Parameter:

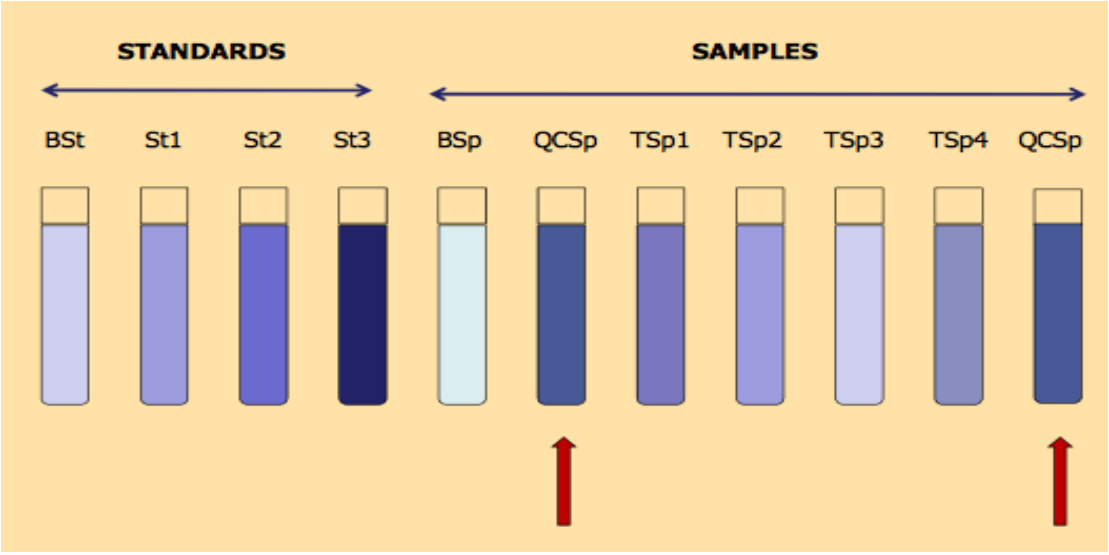
- Limit of detection.
- Limit of Quantitation.
- Precision. (Repeatability, Reproducibility, RSD%, SD)
- Accuracy. (Trueness, Bias)
- Calibration function/linearity.

IV. Analytical Quality Control

The dual functions of the laboratory quality control program ***(Appendix 28** – quality control program***)*** is itsinternal quality control, composed of day-to-day monitoring of analytical performance, and its external quality control, based on the laboratory's performance in proficiency testing schemes and inter-laboratories comparisons.

4.1. Internal Quality Control

- Quality control programs are used to measure accuracy, precision, and matrix effects. Generally, quality control is achieved with each batch. This level sufficiently demonstrates the validity of results. The laboratory determines, where feasible, the accuracy and precision of all analyses performed.
- **Analytical batch –** An analytical batch is the basic unit of measure by which the number of quality control samples needed is determined. The analytical batch is those samples analyzed together with the same method sequence, the same lots of reagents, and manipulations common to each sample within the same time period or in continuous sequential time periods. The frequency of each type of the quality samples is 5% of the batch.



4.1.1.Internal Quality Control schemes for Chemical laboratories

The laboratory shall establish a QC program based on the following elements:

4.1.1.1.Initial Demonstration of Capability (IDC):

To determine the IDC,

- Include a reagent blank and at least four Laboratory Fortified Blanks (LFB) at a concentration between 10 times the Method Detection Limit (MDL) and the midpoint of the calibration curve (or other level specified in the method).
- Undergo precision and accuracy calculations.
- Ensure that precision and accuracy (percent recovery) calculated for LFBs are within the acceptance **criteria listed in the method of choice** or **generated by the laboratory** (if there are no established mandatory criteria).
- To establish laboratory-generated accuracy and precision limits, calculate the upper and lower control limits from the mean and standard deviation of percent recovery for at least 20 data points:
    - Upper control limit = Mean + 3(Standard deviation)
    - Lower control limit = Mean - 3(Standard deviation)
    - Standard Deviation (s) is calculated using the following formula:

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

- Laboratory-generated acceptance criteria for the IDC (in the absence of established mandatory criteria) generally would meet industry-acceptable guidelines for percent recovery and percent relative standard deviation (%RSD) criteria (e.g., 70 to 130% recovery/20% RSD).
- Another option is to obtain acceptance criteria from a proficiency testing (PT) sample provider on the inter-laboratory PT studies and translate the data to percent recovery limits per analyte and method of choice.
- Also, verify that the method is sensitive enough to meet measurement objectives for detection and quantitation by determining the lower limit of the operational range.
- The measurements and calculation of IDC should be recorded in ***(Appendix 29** – internal quality control***)**

4.1.1.2.Method Detection Level Determination and Application:

- Before analyzing sample determine the MDL for each analyte of interest or method to be used:
  - Use an estimate of five times the estimated true detection level. Start by adding the known amount of constituent to reagent water or sample matrix to achieve the desired concentration.
  - Ideally, prepare and analyze at least seven portions of this solution over a 3-d period to ensure that the MDL determination is more reliable
  - The replicate measurements should be in the range of one to five times the estimated MDL, and recoveries of the known addition should be between 50 and 150%, with %RSD values 20%.
  - Calculate the estimated standard deviation, s, of the 7 replicates, and from a table of one-sided t distribution, select t for the (7-1) = 6 degrees of freedom at the 99% confidence level. This value, 3.14, is then multiplied by the calculated estimate of standard deviation, s:

MDL = 3.14 * s

Ideally, use pooled data from several analysts

$$S_{pooled} = \left[\frac{\sum_{i=1}^{N_1} (X_i - \overline{X}_1)^2 + \sum_{j=1}^{N_2} (X_i - \overline{X}_2)^2 + \sum_{k=1}^{N_3} (X_i - \overline{X}_3)^2 + \ldots}{N_1 + N_2 + N_3 \ldots - N_t} \right]^{1/2}$$

- Conduct MDL determinations at least annually The measurements and calculation of MDL should be recorded in ***(Appendix 29 –*** *internal quality control*)**.**

Note: Method Quantification Limit (MQL) is the concentration of an analyte that can be determined with acceptable precision and accuracy under the stated conditions of the method and equal to

MQL=2.5*MDL

- Generally, apply the MDL to reporting sample results as follows:

- Report results below the MDL as “not detected” (ND).

➢ Report results between the MDL and MQL with qualification for the quantified value given.

➢ Report results above the MQL with a value.

4.1.1.3.Operational Range:

- Before using a new method or instrument, determine its operational (calibration) range (upper and lower limits). Use concentrations of standards for each analyte that provide increasing instrument response.
- The measurements and calculation of operational range should be recorded in ***(Appendix 29 –*** *internal quality control***)**

4.1.1.4.Calibration Curves

- For tests that use calibration curves, the following guidance is relevant.

a. Initial calibration:

➢ Perform initial calibration using at least three concentrations of standards for linear curves, at least five concentrations of standards for nonlinear curves, or as specified by the method of choice.

➢ Set the lowest concentration at the reporting limit. The highest concentration standard defines the upper end of the calibration range.

➢ Ensure that the calibration range encompasses the analytical concentration values expected in samples or required dilutions.

➢ Choose calibration standard concentrations with no more than one order of magnitude between concentrations.

- If linear regression is used, use the minimum correlation coefficient specified in the method. If the minimum correlation coefficient is not specified, then a minimum value of 0.995 is recommended.
- Compare each calibration point to the curve by recalculating its concentration. If any recalculated concentration is not within the method's acceptance criteria, identify the source of outlier(s) and correct before sample quantitation. Alternatively, a method's calibration can be judged against a reference method by measuring the method's "calibration linearity" or %RSD among the "response factors" at each calibration level or concentration.

b. Calibration verification:

- In calibration verification, analysts periodically use a calibration standard to confirm that instrument performance has not changed significantly since initial calibration.
- Base this verification on time (e.g., every 12 h or each use) or on the number of samples analyzed (e.g., after every 10 samples).
- Verify calibration by analyzing one standard at a concentration near or at the midpoint of the calibration range.
- Evaluate the calibration verification analysis based either on allowable deviations from the values obtained in the initial calibration or from specific points on the calibration curve.
- If the calibration verification is out of control, then take corrective action, including reanalysis of any affected samples.
- Refer to the method of choice for the frequency of and acceptance criteria for calibration verification.

4.1.1.5.Reagent Blank:

- A reagent Blank consists of reagent water and all reagents included in test except the analyte of interest to determine and measure contamination and interferences.
- Include one reagent blank with each sample batch
- Evaluate reagent blank results for contamination, if unacceptable contamination is present in the reagent blank identify, eliminate, re-prepare and re-analyze samples with a contaminated blank.
- Criteria to accept the result of RB will be< 1/2 MQL.

"If the reagent blank is greater than the MQL, further corrective action and qualification is required. (Appendix 30 – blank determination)"

4.1.1.6.Laboratory-Fortified Blank/Laboratory Control Standard:

- *A Laboratory-Fortified Blank* is a reagent water sample (with associated preservatives) to which a known concentration of the analyte(s) of interest has been added,and is used to evaluate laboratory performance and analyte recovery in a blank matrix.
- As a minimum, include one LFB with each sample set (batch).
- Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve, or level specified in the method.

- Evaluate the LFB for percent Recovery by comparing its recovery results to method specified limits, control charts, or other approved criteria. If LFB results are out of control, take corrective action, including re-preparation and re-analysis of associated samples if required.

$$\% \text{ Recovery} = \frac{\text{Found value}}{\text{True value}} \text{ X } 100\ \%$$

- Results of *LFB* should be recorded in ***(Appendix 31 –*** *laboratory fortified blank*)

4.1.1.7.Laboratory-Fortified Matrix (Spike recovery):

- *A Laboratory-Fortified Matrix (*LFM*)*is a portionof ***sample*** to which a known amount of the analyte (s) of interest is added before sample preparation, and is used to evaluate analyte recovery in a sample matrix.
- Include at least one LFM with each sample batch
- Use an added concentration of at least 10 times the MDL, less than or equal to the midpoint of the calibration curve, or level specified in the method.
- Take certain volume of sample and add certain volume of known concentration standard (i.e. this volume will equal known concentration C_{add})
- Measure the concentration of analyte in the original sample (C_{org}), and in spiked sample (C_{spk}) then Calculate the percent *Recovery*

$$\text{Recovery} = [(C_{spk} - C_{org})/C_{add}] * 100$$

- Evaluate the *LFM* for percent *Recovery* by comparing its recovery results to method specified limits or other approved criteria. If *LFM* results are out of control, take corrective action to correct the matrix effect.
- The recovery data should be recorded in the spike recovery recorded sheet ***(Appendix 32 –*** *spike recovery*)

4.1.1.8.Duplicate Sample/Laboratory-Fortified Matrix Duplicate:

- Duplicate samples are two separate samples taken from the same source (i.e. samples in separate containers and analyzed independently).
- They sample are analyzed randomly to assess precision. If an analyte is rarely detected in a matrix type, use an LFM duplicate.
- As a minimum, include one duplicate sample or one LFM duplicate with each sample batch.

- Evaluate duplicate sample/LFM duplicate results for precision, by Relative percent difference (RPD):

$$RPD = \frac{|\text{sample result} - \text{duplicate result}|}{(\text{sample result} + \text{duplicate result})/2} \times 100$$

- If duplicate sample/LFM duplicate results are out of control re-prepare and re-analyze the sample and take additional corrective action.

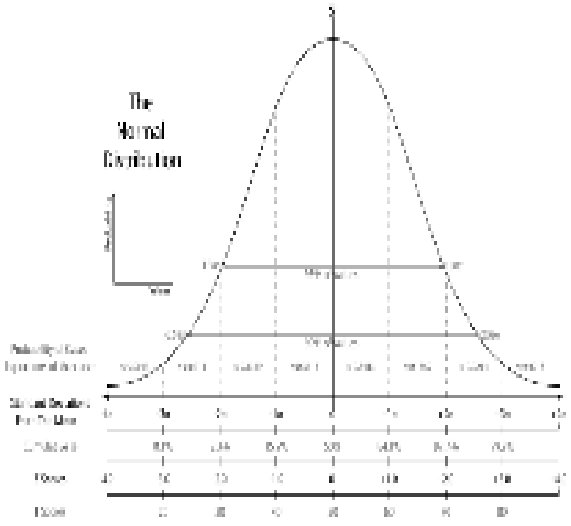
  • The results of duplicate should be recorded in ***(Appendix 33** – duplicate analysis***)**

4.1.1.9.Quality Control Sample QCS:

- Evaluate proficiency for each analyte and method in use by periodically analyzing laboratory check samples. To determine each method's percent recovery, use either check samples containing known amounts of the analytes of interest supplied by an outside organization or else blind additions prepared independently in the laboratory.
- In general, method performance is established beforehand; acceptable percent recovery consists of values that fall within the established acceptance range. For example, if the acceptable range of recovery for a substance is 85 to 115%, then analysts are expected to achieve a recovery within that range on all laboratory check samples and to take corrective action if results are outside it.

4.1.1.10. Control charts

- The **control chart** is a graph used to study how a process changes over time. Data are plotted in time order. A **control chart** always has a central line for the average, an upper line for the upper **control** limit and a lower line for the lower **control** limit. These lines are determined from historical data. Control chart is one of the



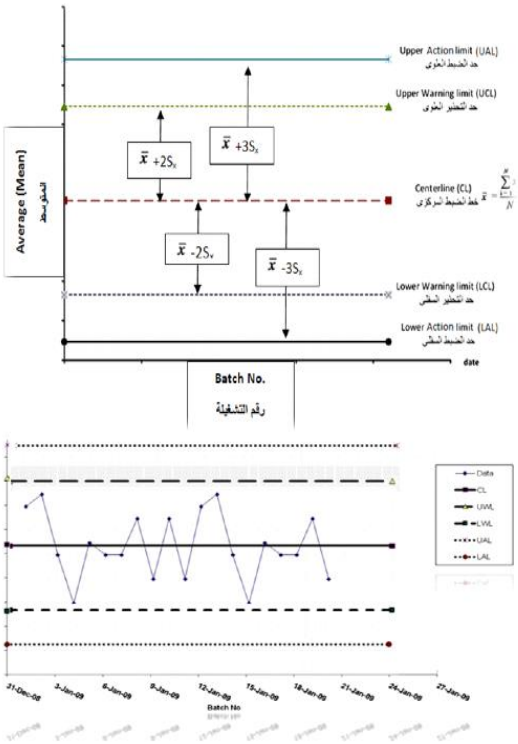
most important tool to monitor the trends of the laboratory's results.

Basically We have to remember the following:

- The Normal Distribution Function
- Standard deviation, Mean
- Confidence intervals, Confidence limits
- Relative Percent Difference
- **Accuracy charts (*Mean Chart/ Recovery Chart*)**
  - Accuracy chart is constructed from the average and standard deviation of specified number of measurements of analyte of interest.
  - The accuracy chart includes:
  - Upper and lower warning levels (WLs) as WL = mean ± 2s (relative to statistical confidence limits of 95%)
  - Upper and lower control levels (CLs) as CL = mean ± 3s (relative to statistical confidence limits of 99%)
  - Central line represents the mean of measurements.
  - Construct a chart for each analytical method, Enter results on the chart each time the QC sample is analyzed.

Control Chart

Accuracy Chart : X-Chart



Batch NO (Date),(Number)	X_1	X_2	$\bar{x}$ $(X_1+X_2)/2$	$\bar{\bar{x}}-\bar{x}$	$(\bar{\bar{x}}-\bar{x})^2$
1-Jan-09	116	116	116	1.625	2.640625
2-Jan-09	116	117	116.5	2.125	4.515625
3-Jan-09	114	114	114	-0.375	0.140625
4-Jan-09	112	112	112	-2.375	5.640625
5-Jan-09	114	115	114.5	0.125	0.015625
6-Jan-09	114	114	114	-0.375	0.140625
7-Jan-09	114	114	114	-0.375	0.140625
8-Jan-09	116	115	115.5	1.125	1.265625
9-Jan-09	113	113	113	-1.375	1.890625
10-Jan-09	116	115	115.5	1.125	1.265625
11-Jan-09	113	113	113	-1.375	1.890625
12-Jan-09	116	116	116	1.625	2.640625
13-Jan-09	116	117	116.5	2.125	4.515625
14-Jan-09	114	114	114	-0.375	0.140625
15-Jan-09	112	112	112	-2.375	5.640625
16-Jan-09	114	115	114.5	0.125	0.015625
17-Jan-09	114	114	114	-0.375	0.140625
18-Jan-09	114	114	114	-0.375	0.140625
19-Jan-09	116	115	115.5	1.125	1.265625
20-Jan-09	113	113	113	-1.375	1.890625

$$\bar{\bar{x}} = \frac{\sum_{i=1}^{N} \bar{x}}{N} = 114.375 \text{ ppm}$$

$$\sum_{i=1}^{N} (\bar{x} - \bar{\bar{x}})^2 = 35.9375 \text{ ppm}$$

$$s = \sqrt{\frac{\sum_{i=1}^{N} (\bar{x} - \bar{\bar{x}})^2}{N-1}} = \frac{35.9375}{19} = 1.375299 \text{ ppm}$$

- Centerline (CL) = Mean = Average = $\bar{\bar{x}}$ = 114.375 ppm
- Upper Warning limit (UWL)= $\bar{\bar{x}} + 2S_x$ = 117.1256 ppm
- Lower Warning limit (LWL) = $\bar{\bar{x}} - 2S_x$ = 111.6244 ppm
- Upper Action limit (UAL) = $\bar{\bar{x}} + 3S_x$ = 118.5009 ppm
- Lower Action limit (LAL) = $\bar{\bar{x}} - 3S_x$ = 110.2491 ppm

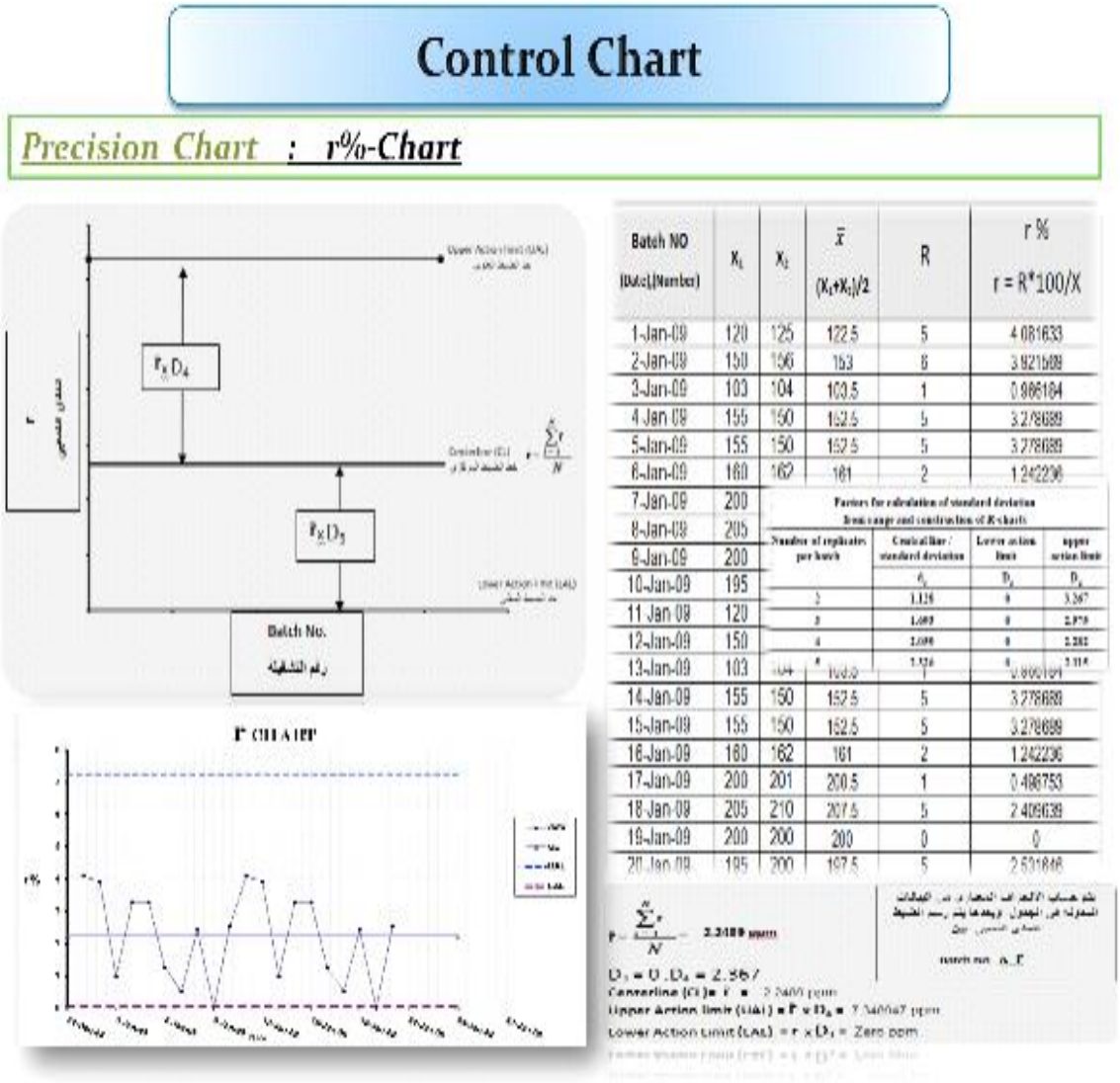
- **Precision charts (RPD, Range Chart)**
  - The precision chart is constructed from the average and standard deviation of specified number of measurement of the analyte of duplicate or replicate analysis of analyte of interest.
  - The precision chart consists of

✓ Upper warning limit $WL = R + 2s\_R = R + 2/3\{(D_4R^*) + (R^*)\} = 2.51^*(R^*)$

✓ Upper control limit $CL = R + 3s(R) = D_4(R^*) = 3.27^*(R^*)$

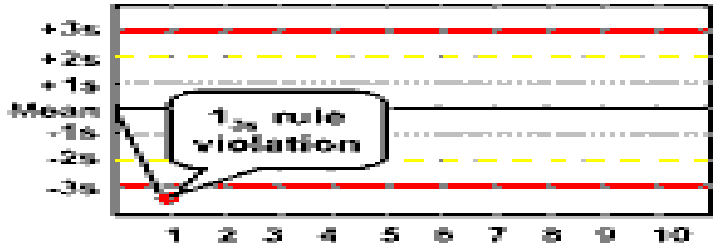
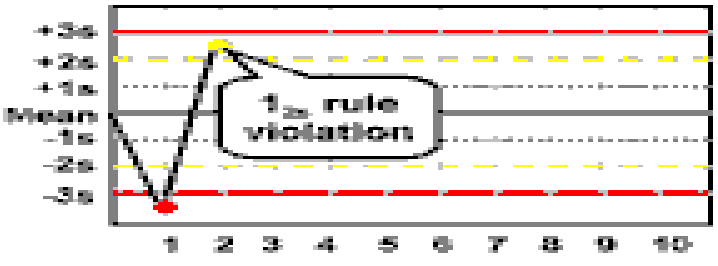
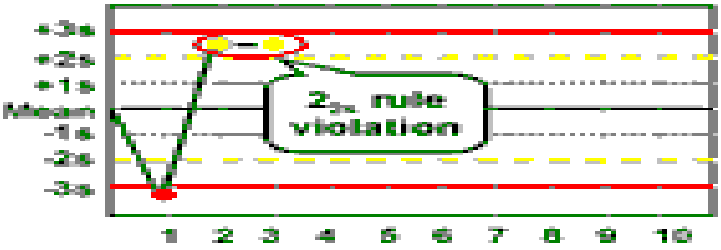
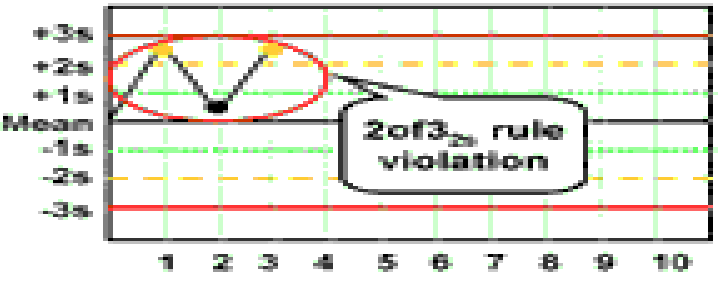
✓ The mean of range $(R^*) = D_2S = 1.28^*S$

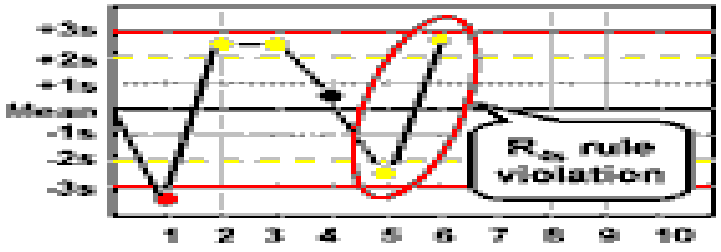
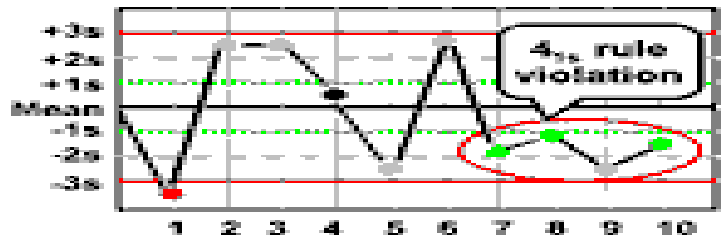
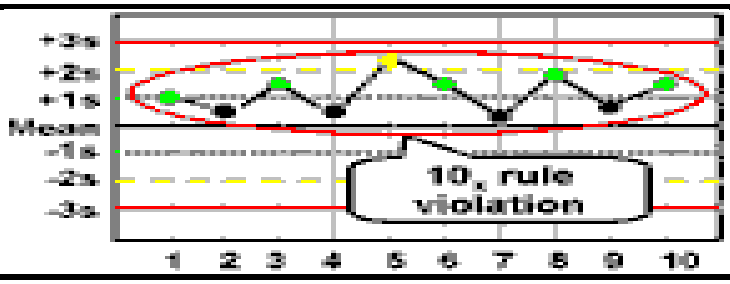
- lower warning limit and lower control limit are the same at zero line .the standard deviation of duplicated readings is converted to the ranges, the analyst need only subtract the two results to plot the value on the precision



chart

- **For evaluation of control chart result and further details for control chart see**

S.N.	Rule	Chart
1	A run is rejected when a single control measurement exceeds the mean plus 3s or the mean minus 3s control limit.	
2	this rule is used as a warning rule to trigger careful inspection of the control data by the following rejection rules.	
3	2_{2s} - reject when 2 consecutive control measurements exceed the same mean plus 2s or the same mean minus 2s control limit.	
4	$2of3_{2s}$ - reject when 2 out of 3 control measurements exceed the same mean plus 2s or mean minus 2s control limit;	
5	7_T - reject when seven control measurements trend in the same direction, i.e., get progressively higher or progressively lower.	

6	R_{4s} - reject when 1 control measurement in a group exceeds the mean plus 2s and another exceeds the mean minus 2s.	
7	4_{1s} - reject when 4 consecutive control measurements exceed the same mean plus 1s or the same mean minus 1s control limit.	
8	10_x - reject when 10 consecutive control measurements fall on one side of the mean	

- Data collected for control chart are recorded in ***(Appendix 34** – control chart collection data*)

4.2. External quality control

- In addition to the internal quality control, it is extremely useful for laboratories to obtain an independent check of their performance and to be able to compare their performance with that of other laboratories carrying out similar types of analyses. Failure to utilize proficiency testing can leave a crucial void in the laboratory quality system. A key feature of proficiency testing schemes is that the assessment of laboratory performance is expressed in terms of a score that can be readily interpreted in terms of statistics.

Calculating Z-score

$$Z = \frac{Result\ of\ indivdual - Mean}{Std\ deviation}$$

The criteria of the z-score results

$-2 \leq Z \leq 2$	*Satisfactory.*
$2 < Z < 3$	*Questionable.*
$Z \geq 3$	*Unsatisfactory.*

V. Appendices

- Look at the attached file, they are ordered as matched with their numbers in context.

VI. References

1. Garfield, F., Klesta, E., Hirsh, J., (2000). Quality Assurance Principles for Analytical Laboratories. 3rd Edition, AOAC International.
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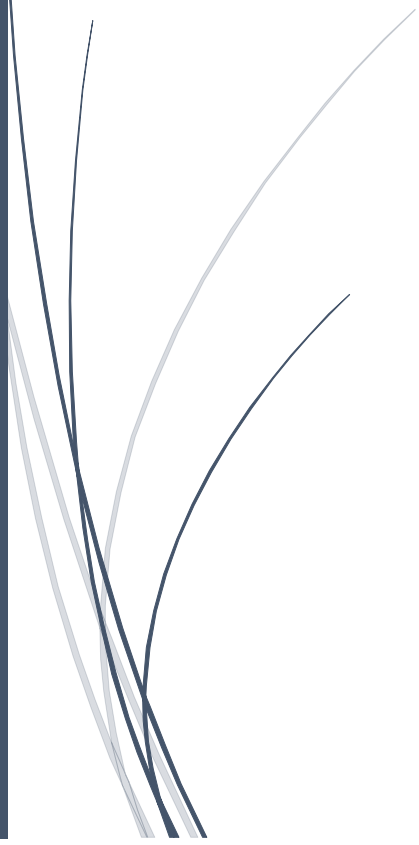
8. Best Laboratory Practices (BLP), 2018, 2nd edition. Fayoum Drinking Water and Sanitation Co.

Part 2 MICROBIOLOGY LABORATORY



I. Introduction:

- Quality control (QC) practices are designed to ensure that the laboratory's processes are under control.
- QC activities are necessary to minimize systematic and random errors resulting from variations in personnel, instrumentation, equipment, reagents, supplies, sampling and analytical methods, data handling, and data reporting.
- A listing of key QC practices is given in Annex 1.

II. QC practices:

- QC practices involving:

1. Personnel and Biosafety Criteria,
2. Environmental Monitoring,
3. Laboratory Equipment and Instrumentation,
4. Laboratory Supplies,
5. Analytical Quality Control Procedures

- Personnel and Biosafety Criteria:

- Microbiological testing should be performed by a professional microbiologist with an appropriate level of education, training, and laboratory bench experience in general microbiological techniques.
- Access to the laboratory is limited or restricted by posting a sign (e.g., "Restricted Area—Staff only").
- Ensure that doorways and windows are closed when aseptic work is in progress.
- Wash hands thoroughly with soap and water after handling viable materials, after removing gloves, and before leaving the laboratory.
- Do NOT eat, drink, smoke or store food for human use in work areas.
- Do NOT pipet by mouth.
- Decontaminate work surfaces before and after each use and after any spill of viable material.
- Decontaminate all cultures, and other regulated wastes before disposal by an approved decontamination method, such as autoclave sterilization.
- Wear laboratory coats to avoid contaminating or soiling street clothes.
- Wear gloves, especially if there is a rash or open lesion on the hands.

Staff Competency:

Definitions:

- **Repeatability:** The closeness of the agreement between successive and independent test results obtained by the same method on portions of the same test sample under the same conditions (apparatus, operator, laboratory and within a short period of time).
- **Reproducibility:** The closeness of the agreement between test obtained by the same method on portions of the same test sample under different conditions
- **Bias:** The difference between the expectation of the test results and an accepted reference value.
- **Trend:** Is a long-term movement in time series data or it is a consistent movement in the same direction over a long period in a time series.

Protocols:

Repeatability (One Analyst results):

- Use overnight broth culture of *any available reference strain* to perform this test.
- Pick up one colony from working culture and inoculate in 100 ml tryptic soy broth.
- Incubate at adequate temperature for 12-18 hr.
- Perform 10^{th} fold serial dilutions series using phosphate buffered water as diluents.
- Plate out dilutions number 10^{-7}, 10^{-8}, and 10^{-9} onto non selective agar media plates in duplicate manner.
- Repeat the step of serial dilutions five times.
- Incubate all the plates at adequate temperature for 22-24 hours.
- Transform final result to $\log_{10}$ values and calculate repeatability (r) from equation:

$$\mathbf{r = 2.8 \times Sr}$$

Reliability of Colony Counting (Within Analyst):

- Select, from any available source, 10 bacterial plates cultured with pour/surface plate technique and 10 bacterial plates cultured with membrane filtration technique.
- Mark each plate with unique number.

- Count each plate twice and consider readings as T1 and T2.
- Keep all plates at 5 ±3.0 °C overnight.
- Recode plates with different numbers.
- Recount all plates and record counting as T3.
- Calculate mean value for each plate (T)
- Use following equation to calculate relative difference value for each plate between the three counting results.
- Value should be within ±7.7 %.
- Equations:

[(T1-T2) x 100] / T , [(T2-T3) x 100] / T, [(T1-T3) x 100] / T

Precision Criteria for duplicate analysis:

- Calculate precision, for each analyst, of duplicate analyses according to the following procedure:
- Perform duplicate analyses for at least 15- 20 sets.
- Record duplicate analyses as D1 and D2.
- Calculate the logarithm of each result. If either of a set of duplicate results is <1, add 1 to both values before calculating the logarithms.
- Calculate the range (R) for each pair of transformed duplicates as the mean of these ranges.
- Thereafter, transform the duplicates to $\log_{10}$ and calculate their range. If the range is greater than 3.27 R, there is greater than 99% probability that the analyst variability is excessive.
- Identify and resolve the analytical problem before further analyses.
- Update the criterion used annually by repeating the procedures using the most of duplicate results.
  - Example:

Sample NO.	Duplicate analyses		Logarithms of counts		Rang of Logarithms (R_{log})
	D1	D2	L_1	L_2	(L_1-L_2)
1	89	71	1.9494	1.8513	0.0981
2	38	34	1.5798	1.5315	0.0483
. . .	. . .	. . .	. . .	. . .	. . .
14	7	6	0.8451	0.7782	0.0669
15	110	121	2.0414	2.0828	0.0414

- **Calculation**
  - $\overline{R} = \sum \text{of } R_{log} / n$ Precision Criterion = $3.27\ \overline{R}$

- $\overline{R}$ = 0.71889 / 15= 0.0479…. criteria = 3.27 x 0.0479 = 0.1566

- **Application**:

Date of analysis	Duplicate of analysis		Duplicate of count		Logarithms range	acceptance
	D1	D2	L1	L2		
DD/MM/YY	71	65	1.8513	1.8129	0.0383	A
DD/MM/YY	110	121	2.0414	2.0828	0.0414	A
DD/MM/YY	73	50	1.8633	1.6990	0.1643	U

- Other measures can be used to calculate the difference such as root difference, standard deviation(s), and coefficient of variation (CV).
- Root difference is the difference between two values after taking their square root.
- Coefficient of variation is the division of Standard Deviation (S) by mean (S/M)
- Lab should establish criteria based on previous measures and graph them using R chart.

- Environmental Monitoring:

- Keep work areas clean. Disinfect surfaces before and after testing.
- Regularly clean laboratory rooms and wash benches, shelves, floors, windows, overhead lights, and exposed pipe surfaces.
- Wet-mop floors and treat with a disinfectant solution weekly; do not sweep or dry-mop.
- Wipe benchtops and treat with a disinfectant at least daily.
- Store supplies and paperwork away from benchtops.
- Eliminate or cover any overhead pipes that cannot be cleaned routinely.
- Monitor bench-surface contamination weekly by using swab method
- Check room temperature daily (normally 18 °C – 25 °C).
- Monitor air quality monthly using air-density settling plates (a passive sampling process wherein particles can settle on the agar surface).

- Laboratory Equipment and Instrumentation:

- Identify equipment by serial number or unique laboratory reference number.
- Verify by constant monitoring, routine maintenance and cleaning.
- Provide written procedures on the use, operation, calibration, and maintenance of relevant equipment and instruments.

- Keep manufacturers' manuals available for easy retrieval.
- Use the following QC procedures for all laboratory equipment :

a. Temperature-sensing and -recording devices:

- Use thermometers with temperature increments of 0.5°C or less, as appropriate (e.g., for a 44.5 + 0.2°C water bath used for incubation of thermotolerant bacteria, use a thermometer with 0.1°C increments).
- If using liquid-based thermometers to measure temperatures in air ,incubators and refrigerators, keep thermometer bulb in water or glycerol.
- Annually, verify the accuracy of all working temperature sensing devices .
- Note: Discard temperature-sensing devices that differ by >1°C from the reference device.

b. Balances:

- Check balance routinely before use using at least two working weights that bracket the normal usage range.
- Service and recalibrate balances annually.

c. pH meter:

- To verify that the pH meter is functioning properly, measure and record its slope after standardization each use.

d. Water still (Distiller):

- Each use, monitor it with a standardized conductivity meter.

e. Hot-air sterilizing oven:

- Test performance monthly with commercially available strips of a spore-forming microorganism (e.g. Bacillus atrophaeus).
- Note: Test the strip in glassware similar to the items being sterilized.
- Measure (each use) oven temperature with a thermometer whose bulb is placed in sand, a thermocouple-type probe, or a continuous-read temperature recorder. (The temperature-measuring device must be accurate in the 160 to 180°C range).
- Use heat indicating tape or chemical strips to identify supplies and materials that have been exposed to sterilization temperatures.

f. Autoclave

- When filling the autoclave leave space between racks and flasks so steam can flow past individual test tube racks and flasks.
- Verify autoclave temperature weekly with a maximum registering thermometer (MRT) or accurate high temperature data logger (HTDL).
- Use heat indicating tape or chemical strips to identify supplies and materials that have been exposed to sterilization temperatures.
- Test performance monthly with commercially available strips of a spore-forming microorganism (e.g. Geobacillus stearothermophilus).
- Note: Place the indicator in glassware containing a liquid to simulate actual autoclave sterilization performance on media.
- Each quarter, use a calibrated timer to check the timing of all cycles for media sterilization.

g. Refrigerator

- Maintain temperature at 2 to 8°C and monitor it using either thermometers whose bulbs are submerged in distilled water or glycerol solution.
- Every day while in use, check and record temperature (corrected, if necessary)
- Defrost and clean units annually.
- Volatile organic chemicals should not be stored in the same refrigerator used for microbiological media, reagents, or cultures.

h. Water bath

- Verify that water bath maintain the set temperature,(such as 35 + 0.5°C , 44.5 + 0.2°C or 44°C : 46°C);use an appropriately marked total immersion thermometer.
- When incubator is in use (i.e., samples are being incubated), monitor and record corrected temperature twice daily separated by 4 h.
- When water bath used for tempering of prepared, monitor and record corrected temperature each use.

i. Incubator

- Check and record corrected temperature twice daily (morning and afternoon, separated by at least 4 h).
- Do not overload nor stack Petri dishes more than four plates high.

j. Conductivity meter

- Check daily before use and calibrate, if needed.

k. Microscopes:

- Clean optics and stage and check alignment.

l. Centrifuges:

- Check time of run using calibrated stopwatch quarterly.

- Laboratory Supplies:

a.Reagent-grade water

- Use reagent-grade water to prepare solutions and media, and for final glassware rinses.
- Every day laboratory-prepared reagent water; monitor it with a standardized conductivity meter.
- Each month determine total chlorine residual and heterotrophic bacteria concentrations, which may provide an early warning of potential problems.
- Weekly, biological examination (algae counting & Protozoa) should be done.

b.Membrane filters and pads

- Confirm sterility prior to first use of the lot by placing a membrane filter or a pad saturated with tryptone glucose extract broth (or equivalent non-selective broth or agar) and incubating it at 35 + 0.5°C for 24 h; the filter is sterile if no growth occurs.

c.Culture media

1. Preparation of media:

- Prepare media in clean containers that are at least twice the volume of the medium being prepared.
- Measure both water and media with graduated cylinder or pipets.
- Avoid over boiling by using boiling water bath.

2. Sterilization:

- Do not expose media containing carbohydrates to elevated temperatures for >45 min.
- Do not re-autoclave media.
- After sterilization, check and record pH of a portion of each medium because the specified pH of the medium is the actual pH required for adequate growth. If pH

adjustment is needed, use filter-sterilized 1N NaOH or 1N HCl solutions to make minor adjustments so medium's pH meets that specified in the formulation.

3. Use of agars and broths:

- Temper melted agars in a water bath at < 50°C (preferably 44 to 46°C) until used, but for <3h.

4. Storage of media:

- Prepare media in amounts that will be used within holding time limits given in Table 9020:V
- If media are refrigerated, bring to room temperature before use.

TABLE 9020:V. HOLDING TIMES FOR PREPARED MEDIA

Medium	Holding Time
Broth in screw-cap flasks*	96 h
Poured agar in plates with tight-fitting covers*	2 weeks
Agar or broth in loose-cap tubes*	2 weeks
Agar or broth in tightly closed screw-cap tubes†	3 months
Poured agar plates with loose-fitting covers in sealed plastic bags*	2 weeks
Large volume of agar in tightly closed screw-cap flask or bottle*	3 months

* Hold under refrigerated conditions (2–8°C).
† Hold at <30°C.

d.Glassware &Plastic

- Check Toxicity for each washed batch.
- Check Sterility for each sterilized batch.

- Analytical Quality Control Procedures:

1.1 Membrane Filtration Techniques:

- Filter 10% of sample batch in duplicate manner, final value for that volume will be obtained by getting the arithmetic mean for both plates.
- Plate(s) with non-selective agar medium, labeled as "Environmental Control", will be opened along the sample's analysis run time to ensure adequate environmental conditions.
- Filtration control (Procedural Blank): Filter 100 ml of sterile phosphate buffered rinse water, using separate sterile funnel, after filtration of series of 10 samples;

incubate under the same conditions as a sample. Absence of growth indicates the absence of cross- contamination.

1.2 Multiple Tube Fermentation Technique:

- Apply the presumptive-confirmed phase of the multiple-tube procedure to all samples, if samples were analyzed for fecal coliform using MTF technique there will be no need to apply completed phase, otherwise proceed completed test to not less than 10% of all coliform-positive samples on a seasonal basis.
- Plates with non-selective agar medium, labeled as "Environmental Control", will be opened along the sample's analysis run time to ensure adequate environmental conditions.
- Procedural Blank: perform, under the same conditions as sample, using sterile phosphate buffered dilution water after inoculation of samples series. Absence of growth indicates sterility of inoculation series.

1.3 Pour and Spread Plate Techniques:

- Prepare duplicate plates for each volume of sample or dilution examined; concentration value for that volume will be obtained by getting the arithmetic mean for both plates.
- Plate(s) with non-selective agar medium, labeled as "Environmental Control", will be opened along the sample's analysis run time to ensure adequate environmental conditions.
- Procedural Blank: Inoculate 0.1 mL (for spread plate) or 1 mL (for pour plate) of sterile phosphate buffered dilution water, using separate sterile pipette, after inoculation of samples series; incubate under the same conditions as a sample. Absence of growth indicates absence of cross contamination.

Appendices for calculation of Uncertainty

Appendix 1

WORKED EXAMPLE FOR MEMBRANE FILTER TECHNIQUE

- Let us assume that a drinking water sample was collected and sent to the laboratory for total coliform (TC) analysis by membrane filtration onto mEndo agar LES.
- Let us also assume that the sample was analyzed as soon as it arrived at the laboratory, that there was no significant difference in TC recovery among batches of mEndo agar, that the laboratory had only one incubator for TC and that the total coliform colony count was 0-20 per 100mL after confirmation.
- In this case, there was no holding time, the laboratory had only 1 incubator, there was no significant difference among batches of mEndo agar and the count is in the ranges of 0-20 and 20-80 colonies per filter. So, we only need to include the uncertainty (RSD2) for filtration among analysts and the uncertainty (RSD^2) for counting among analysts for the both ranges to calculate combined standard (u_c) and expanded uncertainty (U).
- **Step 1**
- Calculate the RSD^2 for filtering among analysts. This was 0.0111 (Table 3).
- Calculate the RSD^2 for counting among analysts. This was 0.0071 (Table 4).
- **Step 2**
- Plug the RSDs2 into formula for calculating combined standard uncertainty.
- uc = √ [RSD^2(AMONG ANALYST FILTRATION) + RSD (AMONG ANALYST COUNTING)]
    - **$u_c = \sqrt{(0.111 + 0.0071)} = 0.135$**
- The combined uncertainty as percent relative standard deviations (RSDs%) = 0.135 x 100 = 13.5%.
- **Step 3**
- Calculate the expanded uncertainty (U).
- U (as RSD%) = uc x 2 (as RSD%) = 13.5% x 2 = 27%.
- **Step 4**

- Calculate the final result per reporting volume.
- CFU ± U/100mL = (Count/Filter ± U for the range as RSD%)
- For example: If sample result is 12. In this case, (12 ± 27% of 12) = (12 ± 3) = 12 ± 3 **Therefore, the TC count per 100mL is 12 ± 3 at the 95% level of confidence.**

Table 3: All analysts filtered the same sample and one analyst counted colonies on all filters

Analyst	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter	TC/Filter
A	**19**	**20**	**20**	**17**	**20**	**18**	**17**	**16**	**20**	**19**
B	**18**	**21**	**17**	**16**	**19**	**20**	**20**	**13**	**17**	**20**
C	**19**	**19**	**20**	**15**	**18**	**15**	**19**	**20**	**15**	**18**
D	**17**	**20**	**19**	**18**	**19**	**20**	**18**	**18**	**19**	**13**
Variance	**0.9167**	**0.6667**	**2**	**1.6667**	**0.6667**	**5.5833**	**1.66667**	**8.91667**	**4.91667**	**9.66667**

Overall Mean Count	18.15
Mean Variance	3.6667
Standard Deviation	1.9149
Relative SD %	0.1055
RSD^2	0.0111
Degree of Freedom	30

Table 4: All analysts filtered the same sample and all analysts counted colonies on filters

Count	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate9	Plate 10
A	**19**	**20**	**20**	**17**	**20**	**18**	**17**	**16**	**20**	**19**
B	**20**	**21**	**18**	**16**	**19**	**20**	**20**	**15**	**18**	**20**
C	**19**	**19**	**20**	**18**	**18**	**19**	**19**	**20**	**18**	**20**
D	**17**	**20**	**19**	**18**	**19**	**20**	**18**	**18**	**19**	**13**
Variance	**1.5833**	**0.6667**	**0.9167**	**0.9167**	**0.6667**	**0.9167**	**1.66667**	**4.91667**	**0.91667**	**11.3333**

Overall Mean Count	18.6
Mean Variance	2.45
Standard Deviation	1.5652
Relative SD %	0.0842
RSD^2	0.0071
Degree of Freedom	30

Combined Relative Standard Uncertainty (ru_c)	0.135
Combined Relative Standard Uncertainty (ru_c%)	13.5

Expanded Uncertainty (U %)	27

Appendix 2

WORKED EXAMPLE FOR HETEROTROPHIC PLATE COUNT

- Let us assume that a drinking water sample was collected and sent to the laboratory for HPC analysis by Pour Plate technique onto R2A medium.
- Let us also assume that the sample was analyzed as soon as it arrived at the laboratory, that there was no significant difference in HPC recovery among batches of R2A medium, that the laboratory had only one incubator for HPC and that the HPC colony count was 30 - 300 per 1mL
- In this case, there was no holding time, the laboratory had only 1 incubator, there was no significant difference among batches of R2A medium and the count is in the ranges 30 -300 colonies per 1 ml. So, we only need to include the uncertainty (S_R) for to calculate combined standard (u_c) and expanded uncertainty (U).
- **Step 1**
- Calculate the S_R between Analysts A and B (Table 5).
- Calculate the S_R between Analysts A and C (Table 6).
- Calculate the S_R between Analysts D and B (Table 7).
- Calculate the S_R between Analysts D and C (Table 8).
- **Step 2**
- Multiply all calculated S_R by 2.
- Calculate the Mean 2 S_R
- **EXAMPLE:**
- A standard deviation of reproducibility S_R of ± 0,15 $\log_{10}$ has been found. Thus the expanded uncertainty U, with a coverage factor of 2 (confidence level of 95 %) is 0,15 log.
- The test result is 5,0 log cfu/ 1 ml
- Thus the test result may be reported as one of the following cases:
  - ✓ 5,0 log ± 0.15 log;
  - ✓ 5,0 log [4,85, 5,15].

Table 5: Calculation of S_R between analyst A and B

Sn	A	B	log A	log B	D=A-B	D^2
1	102	140	2.0086	2.14613	-0.13753	0.01891
2	107	141	2.02938	2.14922	-0.11984	0.01436
3	103	137	2.01284	2.13672	-0.12388	0.01535
4	100	136	2	2.13354	-0.13354	0.01783
5	98	139	1.99123	2.14301	-0.15179	0.02304
6	108	140	2.03342	2.14613	-0.1127	0.0127
7	113	141	2.05308	2.14922	-0.09614	0.00924
8	110	137	2.04139	2.13672	-0.09533	0.00909
9	107	136	2.02938	2.13354	-0.10416	0.01085
10	109	139	2.03743	2.14301	-0.10559	0.01115
Sum						0.14252
S_R	0.084416772					
R	**0.238766689**					

Table 6: Calculation of S_R between analyst A and C

Sn	A	C	log A	log C	D=A-C	D^2
1	102	130	2.0086	2.11394	-0.10534	0.0111
2	107	129	2.02938	2.11059	-0.08121	0.00659
3	103	132	2.01284	2.12057	-0.10774	0.01161
4	100	129	2	2.11059	-0.11059	0.01223
5	98	125	1.99123	2.09691	-0.10568	0.01117
6	108	128	2.03342	2.10721	-0.07379	0.00544
7	113	133	2.05308	2.12385	-0.07077	0.00501
8	110	130	2.04139	2.11394	-0.07255	0.00526
9	107	137	2.02938	2.13672	-0.10734	0.01152
10	109	139	2.03743	2.14301	-0.10559	0.01115
Sum						0.09108
SR	0.067485144					
R	**0.19087681**					

Table 7: Calculation of S_R between analyst D and B

Sn	D	B	log D	log B	D=D-B	D^2
1	110	140	2.04139	2.14613	-0.10474	0.01097
2	111	141	2.04532	2.14922	-0.1039	0.01079
3	107	137	2.02938	2.13672	-0.10734	0.01152
4	106	136	2.02531	2.13354	-0.10823	0.01171
5	100	139	2	2.14301	-0.14301	0.02045
Sum						0.06545
SR	0.08090285					

R	0.228827815

Table 8: Calculation of S_R between analyst D and C

Sn	D	C	log D	log C	D=D-C	D^2
1	110	130	2.04139	2.11394	-0.07255	0.00526
2	111	129	2.04532	2.11059	-0.06527	0.00426
3	107	132	2.02938	2.12057	-0.09119	0.00832
4	106	129	2.02531	2.11059	-0.08528	0.00727
5	100	125	2	2.09691	-0.09691	0.00939
6	110	128	2.04139	2.10721	-0.06582	0.00433
7	111	133	2.04532	2.12385	-0.07853	0.00617
8	107	130	2.02938	2.11394	-0.08456	0.00715
9	106	137	2.02531	2.13672	-0.11141	0.01241
10	100	139	2	2.14301	-0.14301	0.02045
Sum						0.08502
SR	0.065199437					
R	0.184411855					

Table 9: Calculation of Mean S_R between analysts and Expanded Uncertainty

	Drinking Water @ 35.0 C	
	S_R	2* S_R
A /B	**0.0844**	**0.1688**
A / C	**0.0674**	**0.1348**
D / B	**0.0809**	**0.1618**
D / C	**0.0651**	**0.1302**
Mean		**0.1489 = 0.15**

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