

برنامج المسار الوظيفي للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

البرنامج التدريبي كيميائي مياه الصرف الصحي - الدرجة الثانية

Quality Assurance/Quality Control



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Quality Assurance in analytical laboratories

ISO 17025 Requirements

3. Management Requirements

1. Organization
2. Management System
3. Document Control
4. Review of Requests, Tenders, and Contracts
5. Subcontracting of Tests and Calibrations.
6. Purchasing Services and Supplies
7. Service to the Customer
8. Complaints
9. Control of Nonconforming Testing and/or Calibration Work
10. Continuous Improvement
11. Corrective Action
12. Preventive Action
13. Control of Records
14. Internal Audits
15. Management Reviews

4. Technical Requirements

1. General
2. Personnel
3. Accommodation and Environmental Conditions
4. Test and Calibration Methods and Method Validation
5. Equipment
6. Measurement Traceability
7. Sampling
8. Handling Test and Calibration Items
9. Assuring the Quality of Test and Calibration Results
10. Reporting of Results

1.0. General:

Setting up a quality system in a laboratory means defining the organizational structure, responsibilities, procedures, processes and resources necessary to achieve the following objectives:

- To prevent risks.
- To detect deviations.
- To correct errors.
- To improve efficiency.
- To reduce costs.

It is essential to have a Quality Manual which lies down formally, concisely and systematically the general principles which should guide the administration (management and operation) of official quality control laboratories to guarantee the quality and integrity of the analytical results, and the associated reliability.

2.0. Quality Manual

Any Quality Manual must include the initial definition of the following basic concepts:

- a. Quality policy.
- b. Objectives.
- c. The responsibility and authority of the areas involved.
- d. General organizational hierarchy in activities relating to quality.
- e. Identification of quality system support documents.

2.1. Quality policy

- The quality policy comprises the guidelines and general objectives of an organization expressed formally by the senior management and supported by the authorities.
- It defines the operating practices, procedures and sequence of relevant activities to ensure the quality of drinking water used. This policy must be supported by a budget allocation to allow its implementation by means of adequate infrastructure and resources and highly trained, specialized personnel.

2.2. Objective of the Quality Manual

The primary purpose of the Quality Manual is to provide an adequate description of the quality system while serving as a permanent reference for the implementation and maintenance of the system. This Manual should be revised continually and updated according to the dynamic evolution of scientific knowledge and technological processes so as to improve the Quality System.

2.3. Responsibility for the Quality Manual

It is the responsibility of the Quality manager of the Laboratory to establish, implement and ensure compliance with the Quality Manual. Quality is the responsibility of all laboratory personnel. More complex laboratories should have a Quality Assurance unit for development of and compliance with the quality program.

3.0. Elements of a Quality Manual

3.1. Organizational structure

3.1.1. Objective

- To integrate the ISO 17025 into the structure of the Quality System and to indicate its relations within this system, and to describe the organization and structure of the Laboratory itself, including the lines of authority and responsibilities.
- In general, depending on the requirements of ISO 17025, a control laboratory must have a management section, an administrative support area, a reception and sample follow-up area, documentation and information management area, technical areas and quality assurance areas. The technical areas can be divided according to the techniques used (microbiology, chemistry, instrumental analyses, biological tests).
- These divisions must never inhibit communication among staff involved in tests on the same sample. The lines of authority should be well defined and communication between divisions must allow a flow of information so that the quality of the samples can be evaluated and judged.
- More complex laboratories require a central office for registration and follow-up of samples which should be responsible for reception of samples with their respective documents, registration, distribution to the testing and

evaluation units, follow-up and monitoring of tests and test results, and final consolidation of the information. Under certain circumstances, some of the functions of Quality Control Laboratories can be performed by laboratories of other national or international institutions. These institutions must not have any conflicts of interest and must be duly accredited and approved by the control organization.

3.1.2. Organogram

The Quality Manual should include an organogram of the Quality Control Laboratory which reflects the hierarchy and lines of authority in addition to the functions and responsibilities of each component of the organogram.

3.2. Staff

3.2.1. Objective

- The control laboratory should have the necessary staff with respect to their number and their qualifications and experience to carry out the corresponding functions and responsibilities.
- Given the characteristics and nature of the work performed by a Control Laboratory, it is important to ensure that all staff involved keep information and results confidential. With the staff are included:
 - a. Director or chief of the laboratory.
 - b. Quality Manager.
 - c. Chief(s) or supervisor(s) of each technical department, section, sector or unit.
 - d. Chief(s) or supervisor(s) of each administrative department, section, sector or unit.
 - e. Laboratory professional, technical and auxiliary staff.
 - f. Administrative support, maintenance, cleaning and service staff.
- Each post must have a job description including: post, functions and responsibilities, academic training required and experience necessary.

3.2.2. Qualifications and Responsibilities of Staff

Director or chief of the laboratory:

The laboratory must be directed by staff of a high professional level, with extensive experience of the existing standards and the analysis of biological products, as well as of control laboratory management.

His/her responsibilities are:

- To establish institutional policies: to plan, program, direct, coordinate and evaluate laboratory activities in order to ensure adequate administration of material and financial resources; and to establish a personnel policy to promote training, continuing education and motivation to participate in the latter, according to cost-efficiency and cost-effectiveness criteria.
- To identify and establish adequate procedures and systems for the purchase and maintenance of facilities and equipment; cleaning; security; and safety at work in general.
- To plan, establish and monitor the work of the laboratory ensuring compliance with the principles of good laboratory practice, including drawing up control programs, and quality and safety guarantees.
- To draw up budgets overall and for the institution's programs according to the applicable legal requirements of the country and to monitor and evaluate the budget management.
- To maintain coordination and cooperation with other national and international units.

Quality Manager

- Ensures that the Quality Management System is established, implemented and maintained in accordance with the requirements of the ISO 17025:2005 standard.
- Manages the internal audit program.
- Coordinates laboratory accreditation activities.
- Handles the maintenance and distribution of the Quality Management Manual and associated documents.
- Maintains a master list of current versions of quality documentation.
- Monitors the Quality Management System.
- Reports on the performance of the Quality Management System to the General Management for review and as a basis for improvement of the Quality Management System.
- Supervises the laboratory's inter-laboratory comparison and proficiency testing programs.
- To advise top management on all matters relating to quality.
- To ensure that the corrective actions arising from audits are discharged within an agreed time scale.
- Prepares regularly management review.
- To review the quality manual and associated documents at least once a year and issue authorized amendment as necessary.
- To be fully responsible of the laboratories procedures index file editing and updating.
- In addition any tasks that may required from Lab General Manager/Technical manager.

Chief or supervisor of a technical department, section, sector or unit

The chief or supervisor of each unit should have the professional training and practical experience in the specific discipline of each sector necessary in order to perform the functions and responsibilities of the unit in his/her charge. He/she has responsibility for leading his/her sector and for the preparation, revision and signature of the final reports of each test and analysis, and other management-related activities.

He/she additionally has the following responsibilities:

- To ensure that the techniques, analytical methods and standard operating procedures (SOP) as well as previously approved and verified protocols are used appropriately in the work of the sector, in order to ensure the quality, integrity and reliability of the results.
- To establish adequate procedures to ensure the quality control of operations and to establish the corresponding corrective actions.
- To ensure that registration of data and results of analyses is in conformity with good laboratory practice, and that the identity of the staff involved in each case can be determined.
- To make sure that the minimal safety and bio-safety conditions for the work are complied with in his/her sector, including accident prevention and treatment (first aid), as well as the appropriate disposal of waste.
- To motivate staff in the application of the principles of good laboratory practice and compliance with safety and laboratory quality control program activities.
- To identify the needs for on-the-job training and continuing education for staff, and to coordinate plans and programs with the person responsible for the specific unit and the Laboratory Chief or Director.
- To work together with the laboratory chief or director and chiefs or supervisors of other units in drawing up plans, programs and projects, and other activities related to the overall management of the institution to help in achieving its objectives and maintaining the highest levels of quality, integrity and reliability of results according to cost-efficiency and cost-effectiveness criteria.
- To ensure the correct use and adequate maintenance of the specific facilities and equipment assigned to the unit.
- To draw up the budget of the unit in his/her charge and monitor budget management.
- To evaluate and monitor his/her immediate subordinates.
- Other duties specifically assigned.

Chief or supervisor of the administrative section, sector or unit

The chief or supervisor of the administrative unit should have the professional training and practical experience necessary in order to perform the functions and responsibilities of the unit in his/her charge.

He / she has, among other things, the following responsibilities:

- To carry out activities related to the accounts management of the budget funds.
- To acquire materials for the laboratory according to current standards.
- To control stock and inventories.
- To manage administrative matters relating to staff and the archiving of staff files and information.
- To work in coordination with the chiefs or supervisors of the other units.
- To coordinate the drawing up of the annual budget according to current standards and to monitor its implementation in coordination with the chiefs or supervisors of the operating units and support services, according to the instructions of the director or chief of the laboratory.
- To direct and coordinate the general support service activities.
- To direct tasks assigned to his/her sector according to the standards and principles governing administrative activities, good laboratory practice and safety.

Laboratory professional, technical and auxiliary staff

The staff performing analytical tasks has responsibility for:

- Carrying out their specific tasks according to standard operating procedures, techniques, analytical methods and analytical protocols previously approved and verified;
- Complying with the internal regulatory standards of the laboratory including those relating to safety, maintenance of equipment and cleaning of implements and rooms, and waste disposal intended to facilitate the laboratory's activities;
- Keeping up to date with the knowledge and preparation necessary for the work he/she is doing, and participating actively in the training and continuing education programs.

4. Documentation

The laboratory shall establish and maintain procedures to control all documents that form part of its management system (internally generated or from external sources), such as regulations, standards, other normative documents, test and/or calibration methods, as well as drawings, software, specifications, instructions and manuals.

In this context "document" could be policy statements, procedures, specifications, calibration tables, charts, text books, posters, notices, memoranda, software, drawings, plans, etc. These may be on various media, whether hard copy or electronic, and they may be digital, analog, photographic or written.

4.1. Document approval and issue

All documents issued to personnel in the laboratory as part of the management system shall be reviewed and approved for use by authorized personnel prior to issue. A master list or an equivalent document control procedure identifying the current revision status and distribution of documents in the management system shall be established and shall be readily available to preclude the use of invalid and/or obsolete documents.

4.2. The procedure(s) adopted shall ensure that:

- a. Authorized editions of appropriate documents are available at all locations where operations essential to the effective functioning of the laboratory are performed;
- b. Documents are periodically reviewed and, where necessary, revised to ensure continuing suitability and compliance with applicable requirements;
- c. Invalid or obsolete documents are promptly removed from all points of issue or use, or otherwise assured against unintended use;
- d. Obsolete documents retained for either legal or knowledge preservation purposes are suitably marked.

Management system documents generated by the laboratory shall be uniquely identified. Such identification shall include the date of issue and/or revision identification, page numbering, and the total number of pages or a mark to signify the end of the document, and the issuing authority (ies).

4.3. Document changes

- Changes to documents shall be reviewed and approved by the same function that performed the original review unless specifically designated otherwise. The designated personnel shall have access to pertinent background information upon which to base their review and approval.
- Where practicable, the altered or new text shall be identified in the document or the appropriate attachments.
- If the laboratory's document control system allows for the amendment of documents by hand pending the re-issue of the documents, the procedures and authorities for such amendments shall be defined. Amendments shall be clearly marked, initialed and dated. A revised document shall be formally reissued as soon as practicable.
- Procedures shall be established to describe how changes in documents maintained in computerized systems are made and controlled.

4.4. Standard operating procedures (SOP)

▪ Objective

To describe in a detailed form the activities performed in the laboratory so as to:

- a. Provide uniformity, consistency and reliability in each of the activities performed in the laboratory;
- b. Reduce systematic errors;
- c. Provide training and guidance for new staff.

▪ Responsibility

Standard operating procedures should be drawn up by specialized technical staff in the operating units, revised by their immediate supervisor and approved by the Director of the institution.

▪ Description

Standard operating procedures should be prepared for general procedures, such as for example:

- **General:** preparation of SOPs, correction of notes and documentation, preparation of protocols, reports.
- **Test systems:** preparation of the areas, maintenance of the areas, sampling method.
- **Laboratory operations:** collection of samples, identification, labelling, washing of material, sterilization of material, storage of samples, labelling of materials and reagents, solutions.
- **Relating to staff:** training, handling of dangerous chemicals, laboratory safety, staffing of each laboratory subunit.
- **Reference materials:** identification, characterization, handling, reception, storage, use.
- **Archives:** maintenance, distribution and updating.
- **Equipment:** calibration, preventive maintenance. For the description of the use and management of equipment, instructions should be used instead of standard operating procedures.

- **Test methods:** methods for processing and analyzing the different samples sent to a laboratory. They should be drawn up according to the following format:
 1. Table of contents
 2. Scope
 3. Principle
 4. Definition
 5. Environment conditions
 6. Interference
 7. Modification to standard test method
 8. Equipment
 9. Chemical and reagents
 10. Precaution
 11. Procedure
 12. Calculation
 13. Quality Control
 14. Method performance data
 15. Reporting
 16. Related documents and records
 17. References
 18. Document History

A Appendices.

A1 Test methods validation/verification protocol.

A2 Measurement uncertainty Budget.

- A simpler format should be used to draw up Operating Instructions, containing title, code and description.
- Each SOP should have the following on each page:
 1. Logo and name of the organization
 2. Department or unit issuing the standard operating procedure (SOP).
 3. Title
 4. Signature of person who drew up the SOP with date (day, month and year)
 5. Signature of person who reviewed it with date (day, month and year)
 6. Signature of person who authorized it with date (day, month and year)
 7. Duration of validity

8. Date of review
9. Code
10. Page number and total number of pages in the document.

5. Accommodation and environmental conditions

1. The laboratory must have adequate space that is properly organized so that the quality of work and the safety of staff, patients, customers and visitors are not compromised. Measures must be taken to ensure good housekeeping (general tidiness, cleanliness, hygiene, freedom from rodents and insects), and maintain all work areas well. Laboratory section leaders should arrange equipment and work stations to ensure efficient and convenient workflow.
2. The laboratory must have an appropriate biosafety environment and facilities to safely handle microorganisms belonging to different bio risk levels as per the mandate of the laboratory.
3. Laboratories must be provided with appropriate utilities including clean running water, lighting (natural and artificial), ventilation, electric outlets, back-up power (if required), drainage systems that comply with environmental regulations, and sanitation facilities for patients and staff.
4. Where primary sample collection is carried out, consideration must be given to patient access (including patients with disabilities), comfort and privacy. Separate rooms should be available for sample collection and blood donor activities.
5. Potentially hazardous activities must be carried out in a separate area to prevent cross-contamination and reduce potential safety risks to all staff and visitors. Examples include: TB bacteriology, handling and examination of high-risk samples, nucleic acid amplifications, and controlled environments for large computer systems and some high-capacity analyzers.
6. Adequate storage space with the right conditions, including refrigerators and freezers, must be available and protection from light, damp, dust, insects and vermin ensured to maintain the integrity of samples, slides, histology blocks, histology samples, retained microorganisms, documents, manuals, equipment, reagents and other supplies, records and results. Storage areas must be adequately secured to prevent unauthorized access.

7. Disposal of all infectious waste including sharps must be managed safely and effectively according to waste management regulations. The laboratory must use separate waste disposal systems for infectious and non-infectious waste. Special containers must be used for sharps disposal, solvents and radiological wastes.

Working areas

5.1.1. Objectives

To describe the minimum technical requirements in terms of working areas for Laboratory in order to allow the flow of staff, equipment, material, samples, reagents, waste and other resources necessary for the work to take place.

5.1.2. Description

General characteristics with which the areas must comply:

- a. A laboratory should be designed according to the technical requirements which will facilitate an adequate flow of staff, material, equipment, samples, other resources necessary for the work and waste, and also complying with the minimum safety requirements to allow the management of potentially dangerous substances, as well as the evacuation of staff if necessary.
- b. The lighting and ventilation should correspond to the needs of each working area, according to the specific requirements of the activity carried out. The surfaces of the work benches should be smooth, easy to clean and made of material resistant to chemicals.
- c. The hot and cold water, treated water, vacuum, gas, steam and electricity installations should be arranged so that they guarantee adequate use during the work and also facilitate maintenance and repair operations. The sewage system should be constructed of a material which ensures its integrity in view of the characteristics of the effluent.
- d. The installations should take into account biosafety standards.

The following working areas are defined:

- Sample reception area

- Area for physicochemical analysis
- Area for microbiological analysis
- Area for biological assays
- Instrumentation area
- Area for washing, preparation and sterilization of materials
- Administrative area
- Storage
- Disposal of contaminating chemical and biological residues
- General services.

5.1.3. Physicochemical analysis laboratory

- There should be areas effectively separated for the performance of tests which require the use of dangerous solvents or radioactive substances or which cause the emission of toxic vapors or gases or release heat, as well as for the preparation of reagents and solutions.
- The work surfaces in the area should be sanitary, with the necessary ventilation and protection against direct sunlight.
- There should be extraction hoods and the necessary safety equipment (masks, goggles, aprons, acid-resistant gloves).

5.1.4. Microbiological analysis laboratory

- There should be an area for the preparation and distribution of culture media or a service for the supply of these.
- The walls, floors and ceilings should be smooth and easily cleaned. The joints between walls, between walls and floor and between walls and ceilings should have sanitary finishes.
- Where necessary there should be an area for the maintenance and growth of test microorganisms and a room for incubators.
- There should be a sterile area for the performance of the sterility test, with a laminar flow cabinet.

5.1.5. Area for biological assays

The design and atmospheric conditions will depend on the assay to be performed and the risk involved in the work. There should be the following working areas:

- Area for the control of bacterial: a Class II safety cabinet is required at least.
- Area for the control of viral: a Class II safety cabinet is required at least.
- Area for the control of human: a Class II B3 safety cabinet is required at least.
- To be able to effectively work with light sensitive organisms, these safety cabinets should allow for working under low light conditions.
- If other biological products are controlled within the laboratory (blood derivatives, cytokines, hormones, biotechnology products), there should be areas for these assays.

5.1.6. Instrumentation area

There should be a specific centralized area for the installation and use of specialized analytical instruments, with controlled relative humidity and temperature and voltage stabilizer.

5.1.7. Area for washing, preparation and sterilization

All the conditions necessary for performing the activities of washing, preparation and sterilization of materials should be met. There should be autoclaves, ovens, and adequate air exhaust systems.

5.1.8. Area for documentation archiving and control

The processing and archiving of the documentation (SOPs, manuals, instruction sheets, registrations) should be carried out, ensuring their confidentiality and allowing their periodic revision and distribution.

5.1.9. Storage

Reagents, culture media and other materials should be stored in areas separate from the testing laboratory, taking special care with inflammable, toxic and radioactive fluids and solids. Air exhaust systems and protection against vectors should be installed and temperature and relative humidity should be controlled in required areas.

6. Equipment and instruments

6.1. Objective

- The laboratory should have the necessary equipment and instruments for the correct performance of the tests. New instruments and equipment should be installed and calibrated by the distributor who should leave a written report of the visit as part of the dossier. The system is established to ensure correct functioning and to maintain the service record. The equipment selected must be of known reliability and the requirements of laboratories at each level. It should also be procurable at an acceptable cost. Account should be taken of energy sources needed to run the equipment, requirement for an uninterrupted power supply (UPS), environmental control such as operating temperature, and future disposal (decommissioning).
- Suppliers must provide adequate training for staff in equipment use, care and maintenance; including hospital biomedical engineers who may be required to carry out maintenance must be maintained in a safe working condition, according to the manufacturers' instructions.

6.2. Responsibilities

For all lab staff, they shall be responsible for:

- the inventory and programs for the preventive maintenance, calibration and checking of the equipment or instruments
- Appointing a person responsible for each piece of equipment or instrument, also stating which his /her responsibilities are.

- The laboratory should have a list of equipment and instruments which should include:
 - Equipment Name
 - Equipment ID
 - Model
 - Manufacture
 - Serial Number
 - Supplier Representative
 - Telephone number
 - FAX
 - Date of arrival
 - Authorized Person for Equipment use
 - Calibration Intervals
 - Planned Calibration Time
 - Internal calibration / Verification
 - Equipment location
- A file should be opened for the equipment or instrument which must contain the general data and registration, and preventive or corrective maintenance, calibration and checking reports should be annexed.
- Each piece of equipment or instrument should have its operating manual in the local language. The operating instructions should describe in a general manner the steps to follow for the use of the equipment and should be kept in a visible place near the equipment.
- Each piece of equipment should have its registration of use and control card kept close by.
- Specific preventive maintenance programs should be established for each piece of equipment, as well as instrument calibration or checking programs to ensure that they operate so that the measurements made are traceable (where the concept is relevant) in relation to national measuring standards and if feasible to those specified by the National Weights and Measures Committee. If the equipment is out of specification, staff should carry out the corresponding corrective actions and in the meantime put up an “out of service” sign. In the case of instruments, it should be demonstrated, through calibration that they are in a satisfactory condition to operate again.
- When equipment is in operation it should undergo in-service checking between periodic calibrations.

1. Equipment should be procured from suppliers who can assure appropriate maintenance and emergency servicing, including availability of spare parts, during the expected life of the equipment. Service contracts must be obtained for major equipment.
2. Suppliers must provide adequate training for staff in equipment use, care and maintenance
3. The ability of suppliers to meet these requirements should be carefully documented and be part of the signed contract or purchase agreement.
4. On installation (commissioning), the laboratory staff must check that the equipment achieves the agreed and specified performance (validation). A record of the commissioning process as well as validation must be kept and subsequent performance must be continuously monitored by the laboratory staff. Validation should be conducted regularly and at least on an annual basis, and as and when maintenance or repairs have been done.
5. Each item of equipment must be uniquely labelled or identified. Documents and records must be kept for each in a safe, specified place and accessible to laboratory staff. Equipment records should include the following:
 - An inventory of all equipment including unique identifying number, manufacturer's name, instrument type and serial number.
 - Whether new, used or reconditioned; date received and date put into service.
 - Manufacturers' or suppliers' contact address, telephone number and/or e-mail address;
 - User and service manuals provided by the manufacturer in a language that is understood by the users;
 - SOPs on how to use the equipment. These must be appropriately placed and routinely updated. Where equipment calibration involves correction or calculation factors, these procedures must be included in the SOPs.
 - Ongoing records of equipment performance criteria (quality control records);
 - Written instructions outlining the steps for cleaning and maintenance, and what to do in case of damage, malfunction, modification or repair;

- Records of all maintenance and repair procedures;
- Availability of spare parts with instructions for future ordering;
- Disposal of redundant/unusable equipment.

3. External calibration contractors

- Make an agreement with the contractors to supply written reports of calibrations, which should include the following:
- Use of standards and references traceable to national standards
- Certification / licensing by the equipment manufacturer, if available
- Checking of all certificates or reports supplied by approved external laboratories on receipt.

7. Methods

7.1. Objective

- These are the appropriate technical procedures for determining one or more specific characteristics of a product or material compatible with the nature of the sample to be tested. They should be available in manuals of methods or written as individual
- SOPs in a clear and precise form, so that an analyst can use the procedure and interpret the results. The SOPs for the methods should follow the prescribed format but should also include the following information: basic principles, equipment and reagents, calculations, statistics and references.

7.2. Selection of methods

There are four principal options for the selection of methods:

- Standard methods:** these are methods which have been the subject of intensive investigation by many individuals and laboratories and have been demonstrated to be the best existing methods, even though they may be old. These methods have been exhaustively tested and validated.

- b. Official methods:** these are methods which are required to be used by laboratories by government regulation, or international organizations (WHO). These methods have also been appropriately validated prior to being designated official methods.
- c. Literature methods:** methods in specialized journals which provide a good source of new methodologies and techniques, but which should be treated with caution and need to be validated exhaustively before they are implemented.
- d. Methods developed internally:** these are methods developed and modified in laboratories as a result of investigations to improve or perfect tests or to meet individual needs and problems. They require validation.
- In spite of the fact that standard and official methods have been exhaustively validated, a quality control laboratory which desires to introduce one of these for the first time should perform validation tests to guarantee that the performance is satisfactory.
- Any new method or one involving major modification of an existing method considered for routine use should be the subject of a rigorous selection process which includes validation.
- **The following questions should be addressed:**
 - a. Does the test satisfy the required characteristics of sensitivity, reproducibility, accuracy and precision?
 - b. Is the adequate instrumentation available?
 - c. Does the laboratory have the experience required to implement the test rapidly or is staff training necessary?
 - d. How expensive is the test?

7.3. Validation of the methodology

The analytical method should be appropriate for answering the questions addressed and should be reproducible with reliable results. The test should incorporate the following characteristics.

- a. Limit of quantitation (LOQ)/lower limit of quantification (LLOQ):** The **smallest** measured content, which it is possible to quantify the analyte with an acceptable level of accuracy and precision. In some laboratories

- b. Accuracy:** degree of correlation with the true value;
- c. Precision:** the variation of the results as represented by the standard deviation or the coefficient of variation;
- d. Sensitivity:** the response per unit of the substance being measured; capacity of the test procedure to record small variations between concentrations;
- e. Reproducibility:** the precision of the procedure when it is performed under different conditions;
- f. Specificity:** the degree of uniformity of the response to the substance in question;
- g. Robustness:** ability to provide accurate and precise results under a variety of conditions.
 - Furthermore, a comparative study should be performed in parallel with the method used routinely to determine the correlation between the two methods, which should be linear. The most acceptable validation procedures for new methods are collaborative studies between laboratories.

h. Reference material

This comprises preparations used to calibrate the test procedures and to guarantee uniformity in determining activity. It corresponds to international standards for biological substances, international reference reagents, working standards and national reference reagents.

1. Purchasing, reception and distribution must be the responsibility of a qualified professional.
2. A central registry or archive should be kept containing the following:
 - name of the reference material;
 - supplier or importer;
 - origin;
 - lot;
 - date of analysis to determine whether it complies with the stipulated requirements (analytical protocols received or analyses performed in the laboratory); in this case, the archive should include the results of all the tests and determinations used to establish the standard and the initials of the analyst responsible; any material discarded should be clearly identified and destroyed or returned to the supplier as soon as possible (corresponding SOP);
 - place and conditions of storage;

- expiry date, where applicable;
 - storage in an appropriate form (corresponding SOP);
 - This registry should contain all the information relating to the properties of the reference material.
3. The quality of the reference material should be verified when the conditions have been altered and routinely once a year. The program for establishing a Regional Reference Material (or other standards and reference reagents) should be prepared from an approved protocol and carried out with an international collaborative study with statistically validated values.

i. Reagents

Definition: these are materials of chemical or biological origin used in laboratory analyses.

1. Characteristics of reagents

- They should be of appropriate quality;
- They should be purchased from certified suppliers in their original packaging;
- They should be the responsibility of a trained technician or professional;
- A record should be kept of purchasing, reception and distribution to guarantee continuity, above all as regards substances which need to be acquired in advance;
- They should be inspected to ensure that the seals are intact when received in the stockroom or when distributed to divisions or units. These inspections should be recorded with the initials of the person responsible for the inspection and the date on the label.
- Reagents appearing to have been tampered with should be discarded, except in cases where their identity and purity can be confirmed;
- There should be a specific SOP for the transport, storage and handling of reagents and the disposal of chemical waste.
- There should be separate, adequate areas for inflammable substances, acids, substances which produce emissions and other reagents.
- All storage areas should be equipped in accordance with fire safety standards.
- They should not be moved from one division or unit to another.

- Repackaging should be avoided.
- In order to promote safety and to reduce laboratory contamination, reagents should not be stored in the laboratory unless there are good reasons to do so. Reagents used routinely should be kept in the laboratory.
- Water should be considered a reagent and should have suitable specifications or other technical requirements for use in the laboratory.
- Reagents prepared in the laboratory should be prepared in conformity with written procedures and where applicable according to other official texts and labelled appropriately, stating the following:
 - identification of the reagent,
 - concentration,
 - normality,
 - preparation and expiry date,
 - storage conditions,
 - Initials of the technician responsible.

2. Microbial strains

- These are standard strains used in the evaluation of microbiological methods. They should be under the responsibility of experienced staff. They consist of pure, stable cultures. Appropriate techniques are necessary to guarantee their viability, purity and stability as regards their genetic characteristics and to maintain them at appropriate temperatures

3. Culture media

- These are preparations necessary for the growth and identification of microorganisms. They should be prepared according to written procedures (SOP) and appropriately labelled. They should be tested to verify that the culture medium promotes growth.

8. Procurement and supplies management

A national selection and standardization process for laboratory supplies and reagents for laboratories at every health-care level is beneficial because it promotes efficiency in inventory control,

storage and distribution; there is better record-keeping and procurement costs are lowered because items are bought in bulk. This involves the following:

- a. There should be documents that define policies and procedures for selection, procurement of reagents and other laboratory supplies, including supplier qualification and monitoring; competitive bidding; ordering based on a reliable estimate of need; and quality checking of supplies received.
- b. A process for validation of supplies, especially diagnostic reagents, should be established.
- c. An inventory of all supplies to avoid stocks-outs of vital supplies should be established. Information including quantities, batch numbers, expiry dates and sources of supply must be recorded.
- d. Lead times between the date of order and receipt should be known to avoid holding excessive quantities of materials that may deteriorate or become out of date during storage, and running out of supplies due to ordering or delivery delays. Orders must be based on an estimation of supply needs and a reasonable buffer stock kept in case of unexpected increase in demand.
- e. Guidelines must be in place to ensure safe and appropriate storage of all laboratory supplies.
- f. Systems must be in place to record dates and batch numbers of individual reagents that are brought into use. Reagents should not be used beyond their expiry date.
- g. Systems must be in place to ensure that the stock is used on a “first in–first out” (FIFO) basis.
- h. A policy must be in place to address donations of laboratory reagents and supplies.

9. Customer service and resolution of complaints

- The laboratory head and authorized staff must be prepared to offer advice to clinical staff and other customers on the use of the service, including operating hours and emergency samples, the types of samples required and interpretation of the results.
- There should be regular meetings between the laboratory head and the users of the service to discuss ways of improving the working of the laboratory.

- A mechanism should be established to document notification to customers when the laboratory experiences delays or interruptions in testing (due to equipment failure, stock-outs, and fall in staff levels, etc.) or finds it necessary to change examination procedures.
- Procedures including documents must be developed for receiving, recording and processing all complaints. Records of complaints, their resolution and minutes of meetings with the users of the service must be recorded and evaluated.

10. Corrective action

- Conduct a review if any measuring device is found to be out of calibration and requires adjustment. Take corrective action where appropriate.
- If the item can be adjusted back into calibration, it may continue to be used. If the item cannot be adjusted back into calibration, it must not be used until the problem is corrected. Under these circumstances, attach an identifying label stating that the item is under repair and is not to be used.
- The supervisor must assess the likely impact of the inaccuracy of the affected measurement on the quality of the current product and that produced since the last satisfactory calibration. Factors influencing the degree of risk include the following:
 - Critical nature of the measurement
 - Sensitivity of quality control testing
 - History of product records and performance checks.
- Additional quality control testing may be instituted to determine whether quality has been compromised. Where it is likely that quality has been compromised, this shall be communicated to senior management and documented in a report.

11. Continuous improvement

Continuous quality improvement (CQI): All operational procedures must be systematically and continuously reviewed by the head of the laboratory to identify potential sources of noncompliance and areas that require improvement. A formal review of laboratory procedures must take place at least once a year.

12. Control of samples

12.1. Objective

To establish a system which guarantees the activities of sampling, reception, storage, distribution to laboratories, follow-up and drafting of the final report by means of standard operating procedures for samples.

12.2. Responsibilities

One person within the laboratory should be appointed responsible for the organization and implementation of this work.

12.3. Description

1. Collection of samples: The collection of samples is the sampling methods and procedures should be described by specific standard operating procedures.

2. Reception of samples: Samples should be received in the area provided for this purpose and they should be accompanied by a test request which should contain the following information:

- Product name
- Lot number
- Lot size
- Control number
- Date of manufacture
- Date of expiry
- Storage conditions
- Size of sample
- Type of tests
- Name, signature, title and date of request
- Observations

3. Purpose of sample: the size of sample and types of tests required should be defined according to the purpose for which the samples were sent to the laboratory, such as lot release, registration, post-marketing surveillance, or quality problems. The size of samples and types of tests required should be

defined according to the purpose for which the samples were sent to the laboratory.

- 4. Registration of samples:** the data corresponding to the samples should be recorded in a registry after a visual inspection, checking that the label is well attached and that the information conforms to that on the accompanying documentation, and that there are no signs of deterioration. The storage temperature at which the sample was received should also be recorded.
- 5. Distribution to laboratories:** the samples should be distributed to laboratories according to the test to be performed accompanied by documentation. Each laboratory in turn should receive the samples and should create an internal reception record for their control.
- 6. Final reports:** the laboratory should organize a follow-up system of the samples and tests to allow consolidation of the results and the issue of a final certificate. These operations must be guaranteed by means of standard operating procedures.
- 7. Waste disposal:** Laboratory staff must have a good knowledge of local waste disposal legislation, and procedures must be strictly followed. Waste materials, including solvents and other organic matter, should be collected and stored in suitable containers, and sent for appropriate, safe disposal or treatment. Liquid waste containing chlorinated solvents must be kept segregated, to avoid potential exothermic reactions and allow separate disposal or incineration. The wastewater from laboratory sinks should pass to an appropriate water treatment system.

13. Internal Audits

To carry out a systematic and independent examination to determine whether the quality activities and their results comply

with the established documentation; to confirm whether these activities are appropriate for achieving the objectives proposed and whether they have been implemented effectively.

13.1. Responsibility

- Audits may be internal, performed by staff who do not have direct responsibility for the areas audited, or by the Quality Assurance department. External audits are performed by official bodies for the accreditation of testing laboratories or by international bodies.
- Audits apply to:
 - the whole quality system;
 - to some elements of the system (procedures, staff, equipment, working areas);
 - processes;
 - products;
 - Services.
- Audits should not be confused with quality control activities or inspections performed for the purpose of process control or acceptance of the product. Laboratories should have an Internal Audit Program.

13.2. Steps in an audit

1. Review of documents
2. Drawing up of an audit plan
3. Opening meeting between auditor and the area to be audited
4. Rapid walk-through of the installation
5. Performance of the audit: interviews, check list, observation
6. Closing meeting
7. Audit report

13.3. Audit report

- The results of the audit, containing the date it was performed, a description of the observations, deviations or instances of non-conformity and the recommendations or corrective measures suggested are compiled into a report.

- This report is sent to the director of the area audited and to the executive director who shall be responsible for ensuring compliance with the resulting recommendations.

13.4. Follow-up audit

If non-conformity is encountered, follow-up audits are performed to verify the implementation of corrective actions.

14. Management Review

It is recommended that a management review of all systems and procedures should be carried out at an agreed frequency, usually once a year. Each review should identify any improvements needed, whether in terms of organization, training, or changes to any particular procedures or analytical methods. These requirements should be documented in an agreed improvement program, with action dates. To facilitate this review, it is useful to identify some key points which might include:

- A judgment of available resources (staff, rooms, sample capacity, etc.)
- Adherence to calibration protocols
- Adherence to maintenance schedules
- Results of proficiency testing programs, if available
- Results from collaborative testing programs.

16. Quality Control

- **Internal quality control (IQC):** The laboratory must perform IQC checks for all relevant tests and procedures. This includes commercial controls (positive and negative samples; quantitative samples in high-, low- and normal ranges; sterility controls) and repeat testing of patients' samples with known results (called "drift controls").
- **External quality assessment (EQA):** Laboratories should participate in inter-laboratory comparisons and national, regional or international EQA schemes. All results must be critically evaluated and used to take corrective action when there is non-conformity or non-compliance.

Standard Calibration Curve LIMIT OF QUANTITATION

- The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.
- The LOQ is generally 5 to 10 times the MDL.

METHODE DITITION LIMIT

- The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte.
- For operational purposes, when it is necessary to determine the MDL in the matrix, the MDL should be determined by multiplying the appropriate one-sided 99% t-statistic by the standard deviation obtained from a minimum of three analyses of a matrix spike containing the analyte of interest at a concentration three to five times the estimated MDL (the lowest Concentration range mentioned in analytical methods), where the t- statistic is obtained from standard references or the table below.

<u>No. of samples:</u>	<u>t-statistic</u>
3	6.96
4	4.54
5	3.75
6	3.36
7	3.14
8	3.00
9	2.90
10	2.82

- **Determine the variance (S) for each analyte as follows:**

$$s^2 = \frac{1}{n-1} \left[\sum_{i=1}^n (x_i - \bar{x})^2 \right]$$

Where:

x_i = the i^{th} measurement of the variable x

$\bar{x}$ = the average value of x

- Determine the Mean ($\bar{x}$) for all analyte as follows

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$

- Determine the standard deviation (s) for each analyte as follows:

$$s = (s^2)^{1/2}$$

- Determine the MDL for each analyte as follows:

$$MDL = t_{(n-1, \alpha = .99)}(s)$$

where $t_{(n-1, \alpha = .99)}$ is the one-sided t-statistic appropriate for the number of samples used to determine (s), at the 99 percent level.

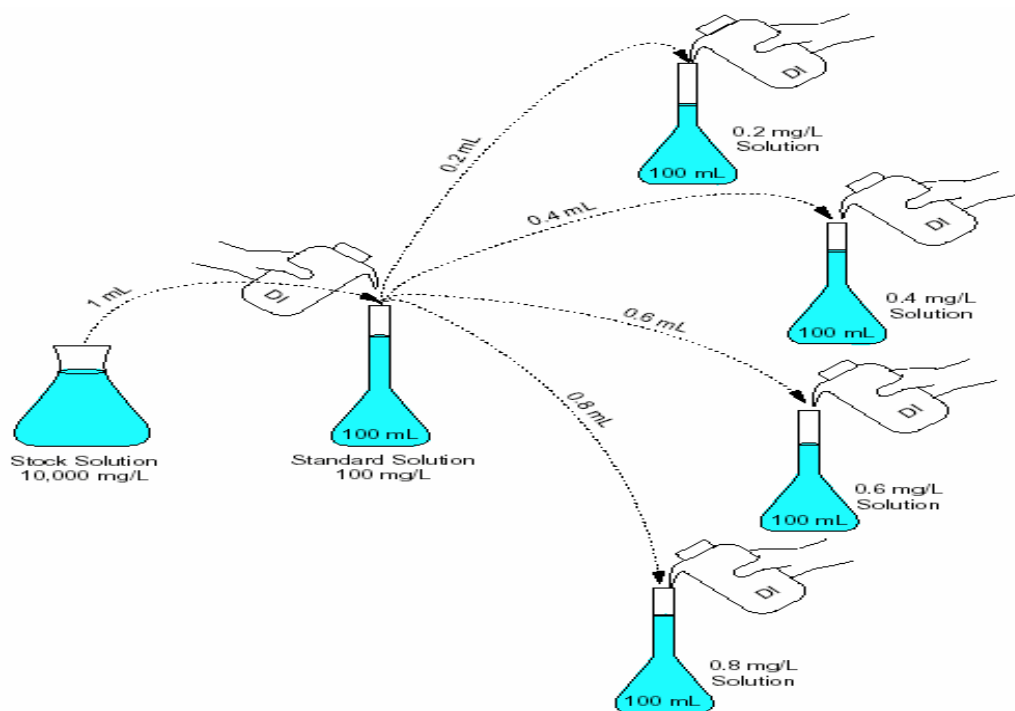
- Calculation LOQ
 - LOQ = 5 time MDL
 - SO LOQ = 5×MDL (which calculate previous)

Cal curve standers concentration

- The first stander use in cal curve is LOQ
- Prepare Cal standards cover your sample Concentration
- Increase the stander Concentration with the factor not more than 2.
- $\frac{3}{4}$ Prefer to use a partial arithmetic series for confine the calibration points concentration, where the concentrations of the upper standards differ by a constant amount, not a constant factor and the lower standers differ by constant factor.
- 10, 20, 40, 80, 120, and 160 mg/L, with a constant difference of 40 mg/L between the top four standards.

To prepare the standards:

1. Prepare the standard weigh dry reagents on a calibrated balance, or measure a solution with a calibrated micropipette, or use a commercially prepared standard.
2. Using reagent-grade water and a standard solution of the compound under examination, prepare at least five standards that encompass the lower and upper concentration range and three equally spaced concentrations in between, plus a reagent blank. Five points cannot be used for some parameters, such as pH (2 or 3 points)



The required sequence to develop the color in each

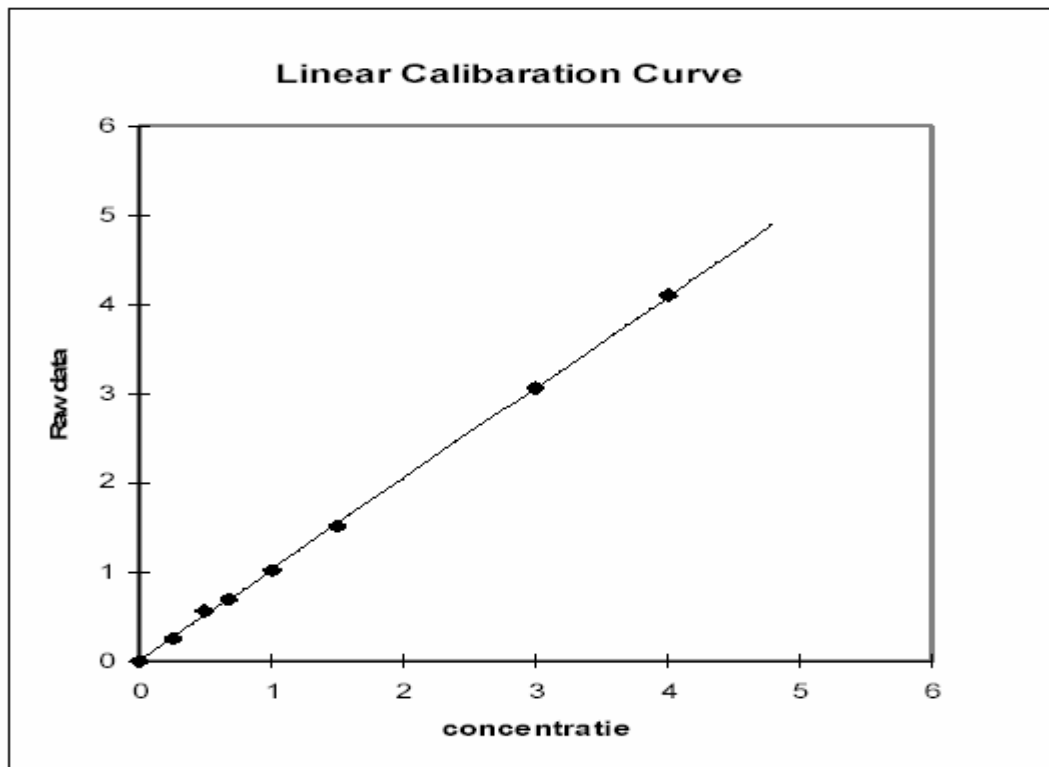
1. Standard. Follow the exact steps performed in the sample analysis. Pay special attention to proper spacing and timing of the standards and samples when color development time is important.
2. Pour an adequate volume of each developed standard into separate, clean, and matched sample tubes, vials, or cuvettes that fit into your colorimetric instrument. Begin with the lowest concentration and move to the highest.
3. Rinse between samples with reagent-grade water, then with a small portion of the standard or sample when reusing cuvettes or vials. The colorimetric instrument should have enough time to warm up and be adjusted to the correct wavelength.
4. Zero the instrument with reagent blank prepared exactly as the standards and samples. Place the sample in the instrument, making sure the sample compartment is tightly closed. Note the absorbance.
5. Plot the results on linear graph program. Plot the absorbance value on the vertical axis (Y-axis) and the concentration on the horizontal axis (X-axis). Plot increasing absorbance values from bottom to top. Plot increasing concentration values from left to right. Values of 0.000 absorbance units and 0 concentrations will begin at the bottom left corner of the graph. Determine an equation for the line using the slope and intercept.

Linear calibration curve Equation

$$Y = aX + b$$

Where

- **a** is curve slop
- **b** is curve interception on Y axis
- **Y** is Absorbance
- **X** is Concentration of Stander used

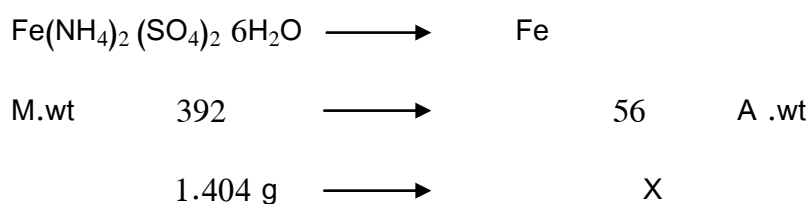


Use this equation to prepare any standard concentration:

$$\text{concentration by mg/L} = \frac{\text{ug}}{\text{sample.volume.(ml)}}$$

1. If stock of iron 1 ml = 200 µg Fe.
2. Prepare standard its concentration 1 mg/l in 50 ml distilled water.
3. At beginning prepare intermediate standard by dill 1 ml from stock solution to 100 ml by distilled water in measuring flask. So 1 ml = 2 µg Fe.
4. To prepare the final stander 1 mg /l take 25 ml from intermediate stander diluted it to 50 ml by distilled water in 50 ml measuring flask.

Example:



$$x = \frac{1.404 \times 56}{392} = 0.2 \text{ g Fe}$$

- Put this weight in 1000 ml distilled water.

So, 1.0 ml of ferrous Amm. Sulfate standard soln. $\equiv$ 0.2 mg Fe

- Complete 5.0 ml of stock soln. to 1000 ml distilled water

So, $5 \times 0.2 = 1.0 \text{ mg Fe}$

The whole soln. of the working soln. contain 1.0 mg Fe

$$1 \text{ ml of the working solution} = \frac{1}{1000} = 0.001 \text{ mg Fe}$$

50 ml $\longrightarrow$ 0.001 mg Fe

1000 ml $\longrightarrow$ X

$$x = 0.001 \text{ mg} \times \frac{1000}{50(\text{volume of the sample})}$$

$$X = 0.001 \times 20 = 0.02 \text{ mg L}^{-1} \text{ Fe}$$

To make the series:

1 mL $\longrightarrow$ 0.02 mg L⁻¹ Fe

5 mL $\longrightarrow$ X, X = 0.1 mg L⁻¹ Fe

10 mL $\longrightarrow$ X, X = 0.2 mg L⁻¹ Fe

15 ml $\longrightarrow$ X, X = 0.3 mg L⁻¹ Fe

20 ml $\longrightarrow$ X, X = 0.4 mg L⁻¹ Fe

Note:

1. Use EXCEL program to evaluate your Cal Curve (Correlation coefficient not less than 0.995)
2. If your sample concentration more than the highest concentration point in your Cal curve dilute your sample before addition the chemicals.

Accuracy, Bias, Trueness and Precision

1. ACCURACY

Accuracy is the closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component and of a common systematic error (or bias) component.

$$\text{Accuracy \%} = (\text{measured Conc.} / \text{Expected Conc.}) \times 100$$

2. BIAS:

Bias is the deviation due to matrix effects of the measured value ($x_s - x_u$) from a known spiked amount. Bias can be assessed by comparing a measured value to an accepted reference value in a sample of known concentration or by determining the recovery of a known amount of contaminant spiked into a sample (matrix spike). Thus, the bias (B) due to matrix effects based on a matrix spike is calculated as:

$$B = 100 (x_s - x_u) / K$$

Where

- X_s = Spike sample
- X_u = unspike Sample
- K = Expected value for spiking stander

3. Trueness

This is expressed by the equation:

$$\text{Trueness\%} = (X / \mu) \times 100$$

Where:

- X = mean of test results obtained for reference sample
- μ = "true" value given for reference sample

4. PRECISION:

Precision is the agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision is estimated by means of duplicate/replicate analyses. These samples should contain concentrations of analyte above the MDL, and may involve the use of:

- a. **Matrix spikes.** The most commonly used estimates of precision are the relative standard deviation (RSD)

$$\text{RSD}\% = 100 \text{ SD}/\bar{x}$$

Where

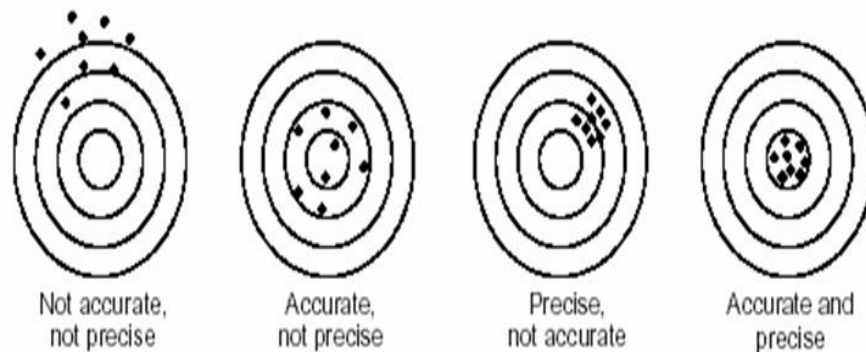
$\bar{x}$ = The arithmetic mean of the measurements.

SD = Stander deviation.

- b. **Relative percent difference (RPD)** when only two samples are available.

$$\text{RPD} = 100 [(x_1 - x_2) / \{(x_1 + x_2) / 2\}].$$

Precision and Accuracy Illustrated



c. Reproducibility

This is a measure of the spread of results when a sample is analyzed by different laboratories. This is a measure of agreement between results obtained with the same method on identical test or reference material under different conditions (execution by different persons, in different laboratories, with different equipment and at different times). The measure of reproducibility R is the standard deviation of these results S_R , and for a not too small number of data ($n < 8$) R is defined by (with 95% confidence):

$$R = 2.8 \times S_R$$

Where: $2.8 = 2\sqrt{2}$ and is derived from the normal or Gaussian distribution.

d. Repeatability

The measure of agreement between results obtained with the same method on identical test or reference standard sample under the same conditions (job done by one person, in the same laboratory, with the same equipment, at the same time or with only a short time interval). Thus, this is the best precision a laboratory can obtain: the within-batch precision. The measure for the repeatability r is the standard deviation of these results S_r , and for not too small number of data ($n > 10$) r is defined by (with 95% confidence):

$$r = 2.8 \times S_r$$

Control Chart

What is a Control Chart?

1. A control chart is a statistical tool used to distinguish between variation in a process resulting from common causes and variation resulting from special causes.
2. Every process has variation. Some variation may be the result of causes which are not normally present in the process. This could be special cause variation. Some variation is simply the result of numerous, ever-present differences in the process. This is common cause variation. Control Charts differentiate between these two types of variation.
3. One goal of using a control chart is to achieve and maintain process stability. Process stability is defined as a state in which a process has displayed a certain degree of consistency in the

past and is expected to continue to do so in the future. This consistency is characterized by a stream of data falling within control limit's based on plus or minus 3 standard deviations (3 sigma) of the centerline.

NOTE:

Control limits represent the limits of variation that should be expected from a process in a state of statistical control. When a process is in statistical control, any variation is the result of common causes that effect the entire production in a similar way. Control limits should not be confused with specification limit's, which represent the desired process performance.

Why should teams use Control Charts?

A stable process is one that is consistent over time with respect to the center and the spread of the data. Control Charts help you monitor the behavior of your process to determine whether it is stable. Like Run Charts, they display data in the time sequence in which they occurred. However, Control Charts are more efficient than Run Charts in assessing and achieving process stability.

Your team will benefit from using a Control Chart when you want to:

- Monitor process variation over time.
- Differentiate between special cause and common cause variation.
- Assess the effectiveness of changes to improve a process.
- Communicate how a process performed during a specific period.

What are the types of Control Charts?

There are two main categories of Control Charts, those that display attribute data, and those that display variables data.

- **Attribute Data:** This category of Control Chart displays data that result from counting the number of occurrences or items in

a single category of similar items or occurrences. These “count” data may be expressed as pass/fail, yes/no, or presence/absence of a defect.

- **Variables Data:** This category of Control Chart displays values resulting from the measurement of a continuous variable.

Examples of variables data are elapsed time, temperature, and radiation dose.

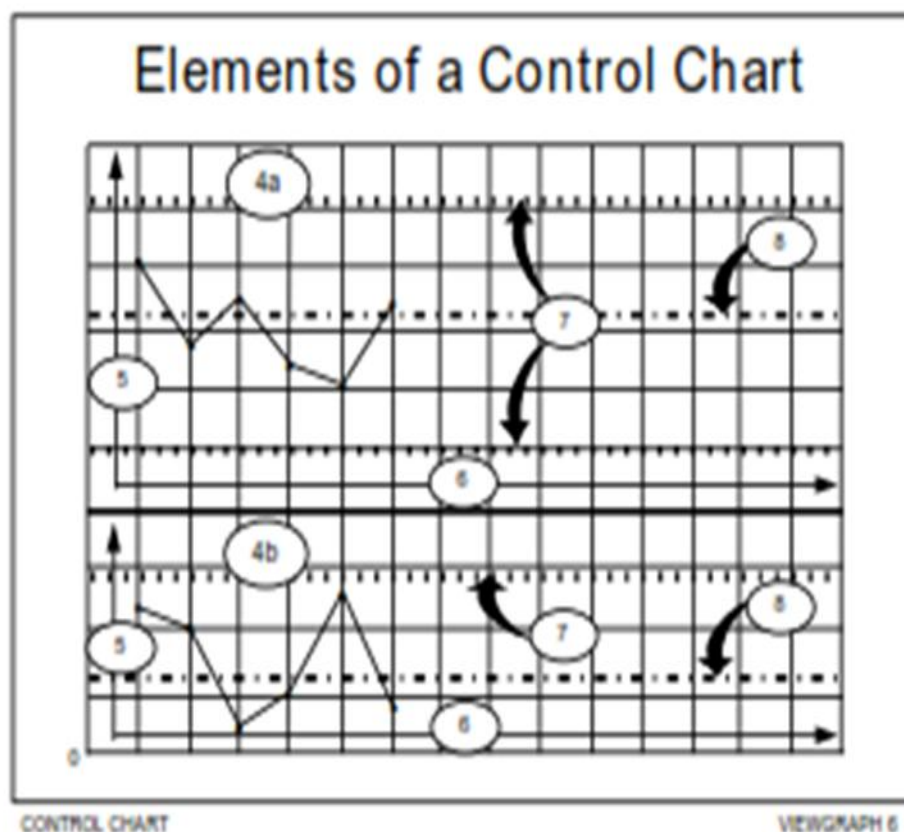
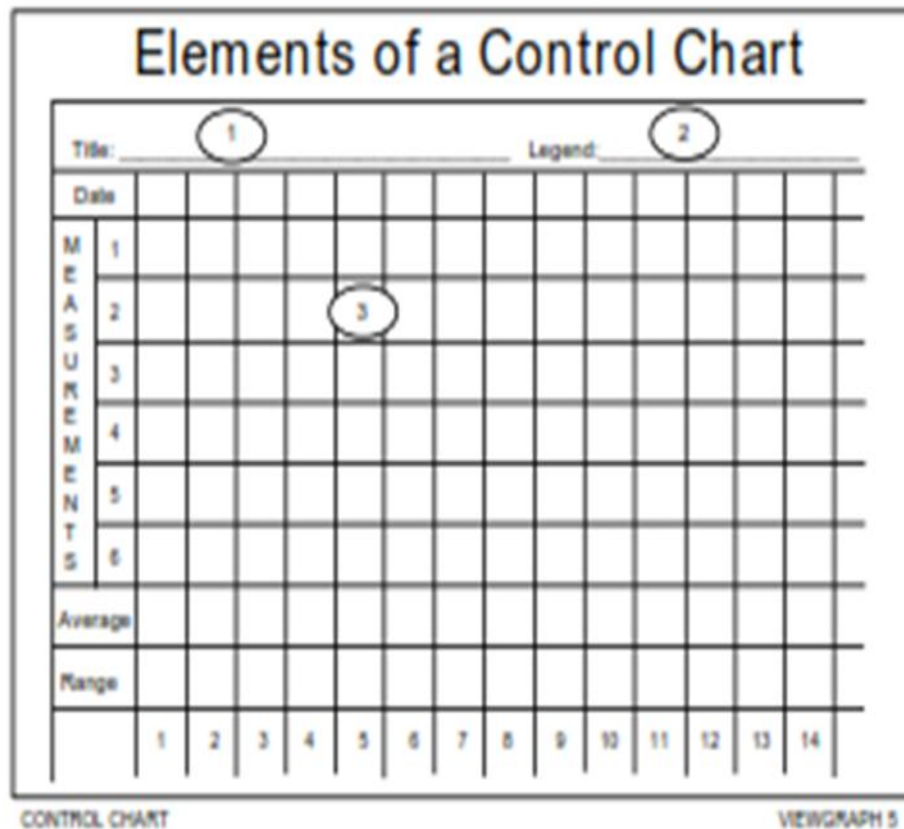
- X-Bar and R Chart
- Individual X and Moving Range Chart for Variables Data
- Individual X and Moving Range Chart for Attribute Data
- X-Bar and S Chart
- Median X and R Chart
- c Chart
- u Chart
- p Chart
- np Chart

What is the elements of a Control Chart?

Each Control Chart actually consists of two graphs, an upper and a lower, which are described below under plotting areas. A Control Chart is made up of eight elements.

1. **Title** The title briefly describes the information which is displayed.
2. **Legend** This is information on how and when the data were collected.
3. **Data Collection Section.** The counts or measurements are recorded in the data collection section of the Control Chart prior to being graphed.
4. **Plotting Areas** A Control Chart has two areas: an upper graph and a lower graph, where the data is plotted.
 - a. The upper graph plots either the individual values, in the case of an Individual X and moving range chart, or the average (mean value) of the sample or subgroup in the case of an X-Bar and R chart.

- b. The lower graph plots the moving range for Individual X and Moving Range charts, or the range of values found in the subgroups for X-Bar and R charts.
- 5. Vertical or Y-Axis.** This axis reflects the magnitude of the data collected. The Y-axis shows the scale of the measurement for variables data, or the count (frequency) or percentage of occurrence of an event for attribute data.
- 6. Horizontal or X-Axis** This axis displays the chronological order in which the data were collected.
- 7. Control Limits** Control limits are set at a distance of 3 sigma above and 3sigma below the centerline they indicate variation from the centerline and are calculated by using the actual values plotted on the Control Chart graphs.
- 8. Centerline.** This line is drawn at the average or mean value of all the plotted data. The upper and lower graphs each have a separate centerline.



What are the steps for calculating and plotting of X-Bar and R Control Chart for Variables Data?

The X-Bar (arithmetic mean) and R (range) Control chart is used with variables data. The steps for constructing this type of Control chart are:

- **Steps 1:** Determine the data to be collected. Decide what questions about the process you plan to answer. Refer to the Data Collection module for information on how this is done.
- **Step 2:** Collect and enter the data by subgroup. A subgroup is made up of variables data that represent a characteristic of a product produced by a process. The sample size relates to how large the subgroups are enter the individual subgroup measurements in time sequence in the portion of the data collection section of the Control Chart labeled MEASUREMENTS (Viewgraph 7).
- **STEP 3:** Calculate and enter the average for each subgroup. Use the formula below to calculate the average (mean) for each subgroup and enter it on the line labeled average in the data collection.

$$\bar{X} = \frac{X_1 + X_2 + X_3 + \dots + X_n}{n}$$

Where: $\bar{X}$ = The average of the measurements within each subgroup
 x_i = The individual measurements within a subgroup
 n = The number of measurements within a subgroup

Average Example

Subgroup	1	2	3	4	5	6	7	8	9
X_1	15.3	14.4	15.3	15.0	15.3	14.9	15.6	14.0	14.0
X_2	14.9	15.5	15.1	14.8	16.4	15.3	16.4	15.8	15.2
X_3	15.0	14.8	15.3	16.0	17.2	14.9	15.3	16.4	13.6
X_4	15.2	15.6	18.5	15.6	15.5	16.5	15.3	16.4	15.0
X_5	16.4	14.9	14.9	15.4	15.5	15.1	15.0	15.3	15.0
Average:	15.36	15.04	15.82	15.36	15.98	15.34	15.52	15.58	14.56

$$\bar{X} = \frac{15.3 + 14.9 + 15.0 + 15.2 + 16.4}{5} = \frac{76.8}{5} = 15.36$$

- **Step 4:** Calculate and enter the range for each subgroup. Use the following formula to calculate the range (R) for each subgroup. Enter the range for each subgroup on the line labeled Range in the data collection section.

$$\text{RANGE} = (\text{Largest Value in each Subgroup}) - (\text{Smallest Value in each Subgroup})$$

Range Example

Subgroup	1	2	3	4	5	6	7	8	9
X ₁	15.3	14.4	15.3	15.0	15.3	14.9	15.6	14.0	14.0
X ₂	14.9	15.5	15.1	14.8	16.4	15.3	16.4	15.8	15.2
X ₃	15.0	14.8	15.3	16.0	17.2	14.9	15.3	16.4	13.6
X ₄	15.2	15.6	18.5	15.6	15.5	16.5	15.3	16.4	15.0
X ₅	16.4	14.9	14.9	15.4	15.5	15.1	15.0	15.3	15.0
Average:	15.36	15.04	15.82	15.36	15.98	15.34	15.52	15.58	14.56
Range:	1.5	1.2	3.6	1.2	1.9	1.6	1.4	2.4	1.6

- **Step 5:** Calculate the grand mean of the subgroup's average. The grand mean of the subgroup's average (X-Bar) becomes the centerline for the upper plot.

$$\bar{\bar{X}} = \frac{\bar{X}_1 + \bar{X}_2 + \bar{X}_3 + \dots + \bar{X}_k}{k}$$

Where: $\bar{\bar{X}}$ = The grand mean of all the individual subgroup averages
 $\bar{X}$ = The average for each subgroup
 k = The number of subgroups

Grand Mean Example

$$\bar{\bar{X}} = \frac{15.36 + 15.04 + 15.82 + 15.36 + 15.98 + 15.34 + 15.52 + 15.58 + 14.56}{9} = \frac{138.56}{9} = 15.40$$

- **Step 6:** Calculate the average of the subgroup ranges. The average of all subgroups becomes the centerline for the lower plotting area.

$$\bar{R} = \frac{R_1 + R_2 + R_3 + \dots + R_k}{k}$$

Where: R_i = The individual range for each subgroup
 $\bar{R}$ = The average of the ranges for all subgroups
 k = The number of subgroups

Average of Ranges Example

$$\bar{R} = \frac{1.5 + 1.2 + 3.6 + 1.2 + 1.9 + 1.6 + 1.4 + 2.4 + 1.6}{9} = \frac{16.4}{9} = 1.8$$

- **Step 7:** Calculate the upper control limit (UCL) and lower control limit (LCL) for the averages of the subgroups. Now, however, the uniqueness of the Control chart becomes evident as you calculate the control limits. Control limits define the parameters for determining whether a process is in statistical control. To find the X-Bar control limits, use the following formula:

$$U/LCL = \text{Mean} \pm 3SD$$

$$U/L\ WL = \text{Mean} \pm SD$$

- **Step 8:** Calculate the upper control limit for the ranges. When the subgroup or sample size (n) is less than 7, there is no lower control limit. To find the upper control limit for the ranges, use the formula:

Use the following constants (D_4) in the computation [Ref. 3, Table 8]:

n	D_4	n	D_4	n	D_4
2	3.267	7	1.924	12	1.717
3	2.574	8	1.864	13	1.693
4	2.282	9	1.816	14	1.672
5	2.114	10	1.777	15	1.653
6	2.004	11	1.744		

Example

$$UCL_R = D_4 \bar{R} = (2.114)(1.8) = 3.8052$$

- **Step 9:** Select the scales and plot the control limits, centerline, and data points, in each plotting area. The scales must be determined before the data points and centerline can be plotted. Once the upper and lower control limits have been computed, the easiest way to select the scales is to have the current.

Plot each subgroup average as an individual data point in the upper plotting area. Plot individual range data points in the lower plotting area

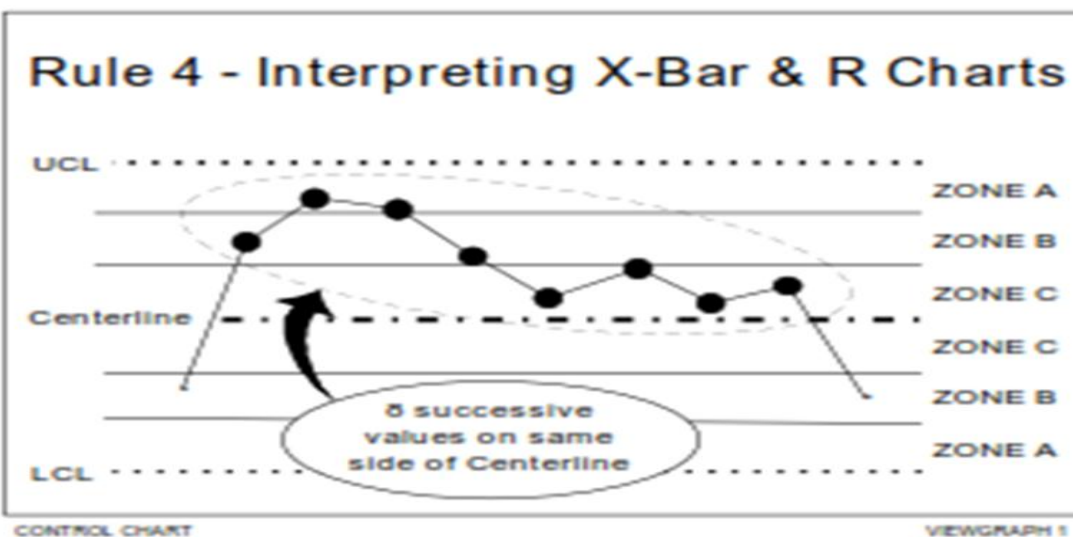
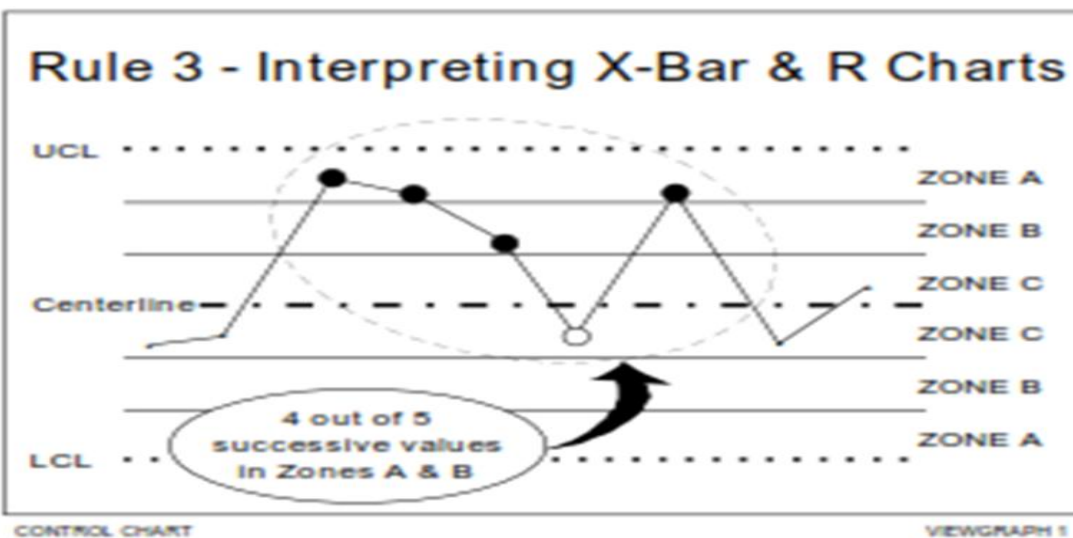
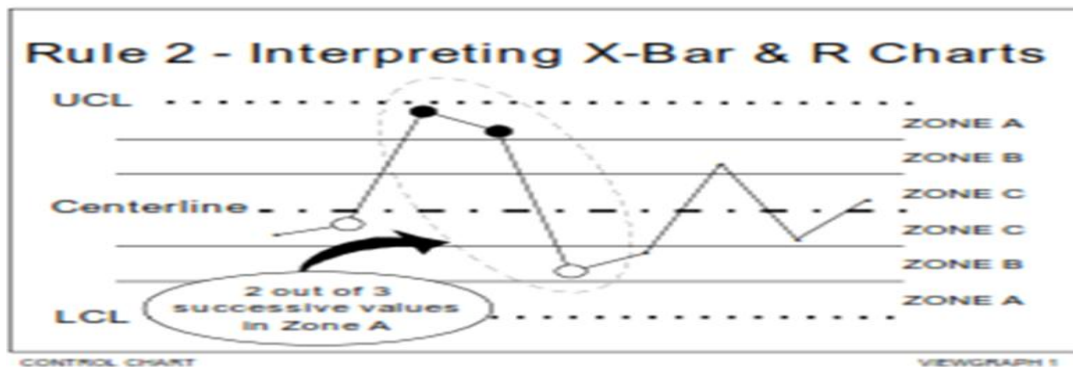
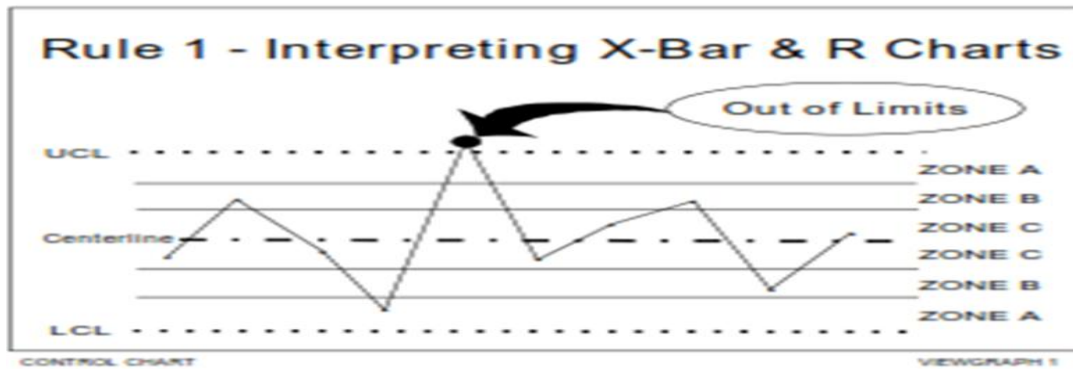
- **Step 10:** Provide the appropriate documentation. Each Control chart should be labeled with who, what, when, where, why, and how information to describe where the data originated, when it was collected, who collected it, any identifiable equipment or work groups, sample size, and all the other things necessary for understanding and interpreting it. It is important that the legend include all of the information that clarifies what the data describe.

What are the rules for interpreting X-Bar and R Charts?

When a special cause is affecting the data, the nonrandom patterns displayed in a Control Chart will be fairly obvious. The key to these rules is recognizing that they serve as a signal for when to investigate what occurred in the process.

When you are interpreting X-Bar and R Control Charts, you should apply the following set of rules:

- **RULE 1:** Whenever a single point falls outside the 3 sigma control limits, a lack of control is indicated. Since the probability of this happening is rather small, it is very likely not due to chance.
- **RULE 2:** Whenever at least 2 out of 3 successive values fall on the same side of the centerline and more than 2 sigma units away from the centerline (in Zone A or beyond), a lack of control is indicated. Note that the third point can be on either side of the centerline.
- **RULE 3 :** Whenever at least 4 out of 5 successive values fall on the same side of the centerline and more than one sigma unit away from the centerline (in Zones A or B or beyond), a lack of control is indicated. Note that the fifth point can be on either side of the centerline.
- **RULE 4:** Whenever at least 8 successive values fall on the same side of the centerline, a lack of control is indicated.



Uncertainty budget

1. Definitions

1.1. Coverage factor

The coverage factor is the number that is multiplied by the standard uncertainty to produce an uncertainty estimate that will contain a large fraction of all values that might be obtained on a test. The coverage factor is commonly noted as k , and $k=2$ is used for 95% coverage, and $k=3$ for 99% coverage.

1.2. Expanded uncertainty

The expanded uncertainty is the combined standard uncertainty (or standard uncertainty, if there is only one component), multiplied by the coverage factor.

1.3. Measurement uncertainty

The measurement of uncertainty is the parameter associated with the result of a measurement that characterized the dispersion of the values that could be reasonably attributed to the measured.

1.4. Measured

The quantity being measured is the measured (i.e. the concentration of an analyte).

1.5. Reproducibility conditions

Reproducibility conditions are identical samples analyzed under different conditions, including any of the following: different times, different equipment, different analysts, or different laboratories.

1.6. S_r

This is a standard deviation or relative standard deviation of result produced under repeatability conditions.

1.7. S_R

This is a standard deviation of results produced under reproducibility conditions.

1.8. Standard uncertainty

The standard deviation for uncertainty is either for the test or for a component of the test. It can be expressed in the units of the measurement, or a percentage, but all components are expressed in the same terms before they can be combined.

1.9. Repeatability conditions

Identical samples prepared at the same time, by the same analyst, under identical conditions, run on the same instruments are repeatability conditions.

2. Procedure

- Measurement uncertainty refers to the ‘uncertainty’ associated with data generated by a measurement process. In analytical chemistry, it generally defines the uncertainty associated with the laboratory process but may also include an uncertainty component associated with sampling.
- The uncertainty ‘estimate’ therefore describes the range around a reported or experimental result within which the true value can be expected to lie within a defined level of probability.

2.1. Estimating uncertainty

- The steps for estimating uncertainty are discussed below. Measurement uncertainty does not usually include uncertainty due to sampling or bias.
- In the inorganic laboratory for the estimation of the measurement uncertainty the “QUALAB” Excel sheet is used. This EXCEL sheet follows the “NORDTEST” approach, described in NORDTEST Report TR 537:2004.
- Using this sheet the operator has to follow the guideline given in the form.
- Starting on the “summary sheet” the operator has to enter some general information about the method.
- After this the operator has to enter on the “reproducibility sheet” some data for the reproducibility component (as relative standard deviation) of the measurement uncertainty estimation. This data may come from:

- X-charts
- OR: Repetitive experiments
- OR: Proficiency test results (Method “B” in NORDTEST)
- In the case the operator is using data from PT (relative standard deviation of all participants) it is not necessary and not allowed to add the uncertainty budget for the bias.
- On the next step the operator has to fill the “bias sheet”. The data for the bias component may be calculated from:
 - X-chart with in-house RM
 - OR: CRM measurement
 - PT results (here the PT is used like a CRM)
- In these cases the “true value”, the found values and the standard deviations have to be entered.
- The calculation is following the NORDTEST approach and the statistical equations listed below.
- As coverage factor $k=2$ is used. This gives a confidence interval of 95% for the expanded uncertainty.

2.1.1 Estimation of measurement uncertainty using relative standard deviation of laboratory control samples run through all method steps:

- a. Perform spiked determinations at different concentrations including tolerance limit.
- b. Calculate concentration and percent recovery.
- c. Calculate the standard deviation (S) and relative standard deviation (RSD) on results where the process is in statistical control (no outliers or out of control results). The uncertainty of measurement could be estimated using RSD from proficiency test as well.
- d. Calculate the measurement uncertainty at the 95% confidence level as follows:

$$U_{\text{expanded}} = k \times \text{RSD}_{(\text{LCS})} \quad \text{or} \quad U_{\text{expanded}} = k \times \text{RSD}_{(\text{PT})}$$

Where:

U_{expanded} = expanded measurement uncertainty

$RSD_{(LCS)}$ = relative standard deviation of lab control sample subjected for within lab reproducibility

$RSD_{(PT)}$ = relative standard deviation from analysis of PT samples.

k = coverage factor (2 for 95% confidence level)

- e. To calculate the measurement uncertainty interval for a measured value, calculate as follows:

$$\text{Test Result Interval} = U_{\text{expanded}} \times C$$

Where:

C = concentration

2.1.2 Estimation of measurement uncertainty using SRW together with method and laboratory bias (IQC + CRM)

2.1.2.1. Analyze lab control sample (LCS) several times and calculate the S_{RW} (Relative Standard Deviation of a set of analysis of LCS analyzed within 1the lab).

2.1.2.2. Convert the S_{RW} into standard uncertainty $u(R_W)$ where $u(R_W) = S_{RW} / 1$

2.1.2.3. Analyze CRM several times n and calculate the bias, S_{bias} as follow:

- Bias= (| nominal value – Average result | / nominal value) × 100
- S_{bias} = RSD of analysis of CRM n times.

2.1.2.4. Calculate the U(bias) using the following equation:

$$U(\text{bias}) = \sqrt{(\text{bias})^2 + (S_{\text{bias}}/\sqrt{n})^2 + U(\text{CRM})^2}$$

N.B Sometimes U(CRM) is not given directly in the certificate of the reference material in that case $U(\text{CRM}) = \text{CRM range } (\pm) / 2$

(e.g. if the CRM is certified to $152 \pm 14 \mu\text{g/kg}$ then $U(\text{CRM}) = ((14 \mu\text{g/kg} / 1.96) / 152) \times 100 = 4.7\%$

2.1.2.5. Calculate the combined standard uncertainty

$$U_{\text{combined}} = \sqrt{(u(R_w))^2 + (u(\text{bias}))^2}$$

2.1.2.6. Calculate the expanded uncertainty

$$U_{\text{expanded}} = 2 \times U_{\text{combined}}$$

2.1.2.7. The measurement uncertainty interval for a measured value, calculate as follows:

$$\text{Test Result Interval} = U_{\text{expanded}} \times C$$

Where:

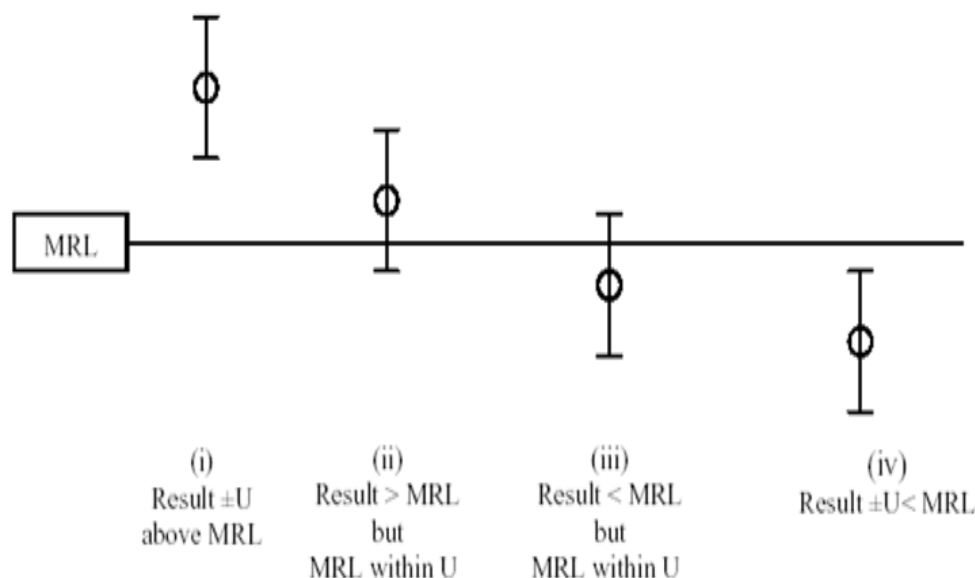
C = concentration

2.2. Expression of uncertainty and testing compliance with an MPL

2.2.1. The test results are reported together with expanded uncertainty as shown in section 3.1.1.5 and 3.1.2.7.

$$\text{Result} = X \pm U_{\text{expanded}} \times C \text{ (mg/kg)}$$

2.2.2. The below shows how the testing results can be displayed in terms of the Measured value of the residue, the corresponding uncertainty interval, and the MPL.



2.2.2.1. Situation (i)

The analytical result bounded by the measurement uncertainty endpoints is greater than the MPL. The result indicates that the residue in the sampled lot is above the MRL.

2.2.2.2. Situation (ii)

The analytical result is greater than the MPL with the lower endpoint of the measurement uncertainty less than the MPL.

2.2.2.3. Situation (iii)

The analytical result is less than the MPL with the upper endpoint of the measurement uncertainty being greater than the MPL.

2.2.2.4. Situation (iv)

The analytical result bounded by the expanded measurement uncertainty endpoints is less than the MPL.

المراجع

• تم الإعداد بمشاركة المشروع الألماني GIZ

• و مشاركة السادة :-

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