

### 3. Guidelines for Quality Assurance in Chemical Pathology

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### 3.1 Introduction

Quality is the degree of congruence between expectation and realization. It is the matching of expectations versus fulfillment. In other words quality means meeting the pre-determined requirements to the satisfaction of the users for a particular substance or a service. The three important cornerstones in healthcare are quality, access and cost which are interdependent. Quality is achieved when health services are accessible to the users and are provided in an efficient and cost effective way.

The essential function of a department of clinical biochemistry /chemical pathology is to provide accurate and reliable data obtained from the examination of specimens taken from patients and to assist in the diagnosis and effective management carried out by the clinicians. Unreliable laboratory results could have serious consequences. It could lead to inappropriate actions such as carrying out over investigations, over or mistreatment of patients. Conversely it could also lead to inappropriate inaction such as under investigation of a disease when actually indicated or not instituting any treatment when required. A poor quality result in the form of delayed reports can lead to delayed actions. All these activities bring about the loss of credibility of the laboratory and in turn invite legal actions against the institution.

Quality Assurance is the sum of total of all activities that are undertaken to ensure generation of reliable and accurate results or data. This is equated with

good laboratory practice starts from test selection, through obtaining a satisfactory sample from the right patient, analyzing it and recording the result promptly and correctly, to appropriate interpretation of and action on the result with all procedures being documented for reference.

Quality control represents the procedures that monitor the performance parameters which will detect the source and magnitude of errors and alert the laboratory personnel of the possible deterioration of quality.

Quality assurance has two components namely, Internal Quality Control (IQC) and External Quality Assessment (EQA). Internal Quality Control is a set of procedures undertaken by the health care professionals in their day to day activities to ensure release of reliable laboratory results. External Quality Assessment is a tool to assessing the implementation of an internal quality control programme and is aimed to improve performance. This is done by an independent agency for inter-laboratory comparisons. This assessment in contrast to IQC is retrospective and periodic. Internal Quality Control programme and External Quality Assessment are mutually complementary.

### 3.2 Essential elements of a quality assurance programme

- ***Commitment***

Dedication to quality service must be central to all personnel involved.

Quality must be a major consideration in all management decisions.

Commitment is required by laboratory directors, specialists, technical and clinical staff.

- ***Facilities and Resources***

Laboratories must have the administrative support necessary to provide the quality services that are desired. This includes having adequate space, equipment, materials, supplies, staffing, supervision and budgetary resources.

- ***Technical Competence***

In order to provide a quality service highly competent laboratory personnel with sound educational background and experience are important as is the capability of providing in-service training that can develop and maintain skills. In-service training can be a mechanism for assuring the competency of laboratory personnel, instilling quality goals, implementing quality control procedures, and providing for the continuing development of laboratory personnel both technically and intellectually.

- **Technical Procedures**

Accurate technical procedures are necessary to provide quality laboratory results. Three groups of procedures are as follows.

1. The pre-analytical procedures or variables such as test requests, patient identification, specimen acquisition and transport, specimen processing and distribution, preparation of work lists and logs and maintenance of records.
2. The analytical procedures or variables which include analytical methodology, standardization and calibration procedures, documentation of analytical protocols and procedures and the monitoring of critical equipment and materials. The monitoring of analytical quality by the use of statistical methods and control charts.
3. The post-analytical procedures or variables such as the preparation of test reports with the inclusion of the decimal points, correct units of measurement, technical and clinical validation and interpretation of test results.

- **Problem solving mechanism**

The mechanism should provide the link between the identification of a problem and the implementation of a solution to that problem. The problems limited to individual methods or instrument systems can be corrected by improving trouble shooting skills and by instituting preventive maintenance. The recurrent problems can be corrected by providing an in-service training, with additional supervision from

a quality control technologist and advice and support from a reference laboratory.

### 3.3 Procedures for monitoring of pre-analytical variables

- **Test utilization**

Laboratory test utilization has been monitored to some degree to provide a cost effective laboratory service. The laboratory will have a role in identifying situations where test utilization can be optimized and in providing in-service education to effect changes in ordering patterns.

- **Patient identification**

The identification on the specimen label should also correspond with the identification on the requisition form. The highest frequency of errors occurs with the use of handwritten labels and request forms. The labeling of the sample containers should be carried out prior to sample collection. The errors may be minimized by the use of a laser beam light wand, which can read barcode or optical characters from labels.

- **Turnaround time**

Lost test requests, specimens and reports are a major problem which can be monitored by recording the actual times of specimen collection, receipt in the laboratory and

reporting of test results. A mechanism should be available to detect the site or the source of the failure in the system. This can be done manually by placing time stamps in key locations or by having computer systems automatically document the times of the flow of test requests

- **Laboratory logs**

In laboratories without computerized reporting, a request form generally accompanies the specimens. One should check that the patient name and identification number and the tests requested on the form match the information on the label of the specimen tube/bottle. The specimen should be inspected to confirm adequacy of volume and freedom from problems that would interfere with the assay, such as lipemia or haemolysis. The specimens are then stored appropriately, and the identification information and arrival time are recorded in a master log. If the analyses are performed in batches, specimen identification generally is recorded in specific locations on the worksheet using the information on the tube labels. After analysis, the results are recorded on worksheet, and if both the assay and the individual test results pass the QC criteria, the test results are transferred to the result forms for reporting. However, prior to the reporting of the results, a second technologist should verify the adequacy of the QC and should check for

transcription errors by comparing the results on the report forms with those on the master log. Specimens that require further analysis because of dilution or assay problems should be indicated either on the master log or on a delayed report log.

- **Transcription errors**

Transcription errors exist in the laboratory even with the double checking of results. Computerization will reduce this error if the patient identification and test results are entered directly from instrumentation that is interfaced to the computer. Manual entry of the results to the computer can still result in errors.

- **Patient preparation**

Laboratory tests are affected by many factors, such as recent intake of food and drugs as well as by smoking, exercise, stress, sleep, posture during specimen collection and other variables. The laboratory must define the instructions and procedures for patient preparation and specimen acquisition which should be included in the procedure manuals.

- **Specimen collection**

The techniques used to acquire a specimen can affect many laboratory tests. Prolonged application of a tourniquet causes local anoxia to cells and excessive back pressure.

The anoxia causes small solutes such as potassium to leak out of cells. The venous back pressure concentrates cells, proteins, and other substances bound to proteins such as calcium. Blood collected from a drip arm may be diluted or contaminated. Haemolysis during and after collection alters the concentration of any analyte that has a red blood cell/plasma concentration differential. Improper containers and incorrect preservatives can affect the test results and make them inappropriate. A specially trained team should be assigned to follow a sample collection procedure manual designed for the laboratory.

- **Specimen transport**  
The stability of analytes during transport is seldom monitored. The sample collection manual should include the requirements of transport and acceptance/ rejection criteria. Fast track mechanisms should be established for the transport and processing of emergency specimens.
- **Specimen separation and aliquoting**  
The centrifuges used for separation should be monitored by checking the speed, timer and temperature. Collection tubes, pipettes and aliquot tubes are sources of contamination by trace metals. The separation of serum should be carried out using individual pasture pipettes or pipette tips to avoid contamination of analytes between samples.

- **Personnel monitoring**  
The personnel who process the specimens should be trained and supervised to follow the written guidelines.

### 3.4 Procedures for monitoring of analytical variables

- **Choice of analytical methodology**  
Reliable analytical methods should be installed in the laboratory by a process of selection, evaluation, implementation, maintenance and control. There are certain variables such as water quality, calibration of equipment, stability of electrical power and the temperature of refrigerators, freezers, water baths and the performance of centrifuges – that should be monitored regularly, since they will affect many of the methods in the laboratory.
- **Reference materials and calibration**  
The reliability of the analytical values obtained with a procedure depends on the quality of the calibrators and calibration procedure.
- **Documentation of analytical protocols**  
Step by step procedures for performing analytical determinations are critical if the methods are to provide the same results when used by different analysts over a long period of time. Maintaining such a consistency requires written protocols or method manuals. These should be reviewed annually and revised when ever changes occur.

The standard operating procedure should include the procedure name, clinical significance, principle of the method, sample collection and specimen type, reagents and equipment, procedure, reference values, comments on any special requirements and references.

- **Verification of reference intervals**

It is recommended that reference intervals be verified for normal subjects representing the population encountered in a respective laboratory.

- **Monitoring of technical competence**

The personnel characteristics and techniques of individual analysts may greatly affect the manual methods. Proper training of personnel in analytical techniques, quality control practices and maintenance of equipment are necessary to achieve quality goals.

- **Inventory control of materials**

Uninterrupted laboratory service depends on the supply of materials that are being used in the methods. Depending on the stability of the materials/chemicals/reagents and diagnostic kits the inventory should be controlled.

- **Maintenance of equipment**

The reliability of test reports depend on the performance of the equipment that are being used for analysis. A standard operating procedure should be developed for all the critical equipment in the laboratory including the preventive maintenance procedures. Calibration of

equipment should be performed at well defined time intervals.

### 3.5 Procedures for monitoring of post-analytical variables

- **Technical and clinical validation**

Acceptability of test results by a process of technical and clinical validation should be carried out by a competent laboratory personnel before the results are released to the patients. During this process the stability of reagents and calibrators, performance of quality control material, units of measurement, the decimal points, age and sex matched reference ranges should be considered for acceptance.

- **Preparation of the test report**

Manual or computer generated reports should include the name, age, sex, hospital number, laboratory reference number, date and time of sample collection, analysis and reporting, relevant reference range and the signature of the authorizing officer should be placed.

### 3.6 Control of analytical quality using stable control materials

The performance of analytical methods can be monitored by analyzing specimens whose concentrations are known and then by comparing the observed values with the known values. The known values are usually represented by a range of acceptable values, or upper and lower limits for control (control limits). When the observed values fall within the control limits, the analyst can be assured that the analytical method is working properly. When the observed values fall outside the control limits, the analyst should be alerted to the possibility of problems in the analytical determination.

#### **Control Materials**

The known specimens that are analyzed for QC purposes are called control materials. They need to be stable materials, available in aliquots or vials, that can be analyzed periodically over a long time. There should be little vial-to-vial variation so that difference between repeated measurements can be attributed to the analytical method alone. The control material should preferably have the same matrix as the test specimens of interest; for example, a protein matrix may be best when serum is the test material to be analyzed by the analytical method. Materials from human sources have generally been preferred, but because there is some risk of hepatitis infection, bovine materials offer a certain advantage in safety and more readily available. The concentration of analyte should be in the normal and abnormal ranges, corresponding to the concentrations which are critical in

the medical interpretation of test results. Control material may be prepared in the laboratory from excess sera. They can be purchased as assayed or un-assayed sera commercially. Assayed materials come with a list of values for the concentrations that are expected for that material. Values may be specified for a reference method.

The commercially available quality control sera are presented in liquid or freeze dried form. A good quality distilled water and volumetric pipettes are required to reconstitute the sample. Following adequate mixing the quality control sera should be aliquoted in to chemically clean vials for immediate freezing. One aliquot may be thawed for an analytical run. Repeated freezing and thawing, exposure to air causing evaporation may deteriorate the quality of the QC sample.

### 3.7 Principals of Internal QC

- **Accuracy:** Closeness of the agreement between the result of a measurement and the true value of the measured

Possible causes of inaccuracy are

- Incorrect sample or reagent volume pipetted
- Incorrect reaction timing or temperature
- Incorrect instrument setting (wavelength)
- Calculation errors
- Deterioration of calibration material

- **Precision:** Closeness of the agreement between independent results of measurements

Possible causes of imprecision are

- Wrong pipetting techniques
- Variable reaction timing and temperature
- Instrument instability (Photometer)

- **Error/uncertainty**

Is a variation in measurement

- **Random error**

Is an unpredictable analytical variation which influence each measurement differently in either a positive or negative direction and to a different extent in magnitude.

- **Systematic error (bias)**

**Constant systematic bias** denotes a constant difference between the true value and the observed value regardless of the concentration level.

**Proportional systematic bias** denotes a difference between the true value and the observed value which changes proportionally as the concentration level changes.

### 3.8 Quality Control Charts and rules

- **Shewhart/Levey and Jennings chart**

Analyze the QC material by the analytical method to be controlled on at least 20 times under optimal conditions and calculate the mean, standard deviation(SD) and coefficient of variation under optimum conditions (OCV%)

Construct the control chart :

Y axis-control value

X axis-days

Mark the mean , +2SD, - 2SD and + 3SD, - 3SD values.

Introduce a control specimen daily to the analytical run, plot each value on the chart.

After 20 days calculate the mean, standard deviation and coefficient of variation under routine conditions.(RCV). Construct the chart, plot daily control values and follow the rules to accept/reject the run.

An analytical run is in control if the daily values are within + 2SD and – 2SD

An analytical run is out of control if one of the following criteria are met.

- A value lies entirely outside the control limits.  
Result > 2SD from the mean- Warning  
Investigate method to avoid future problems  
Result > 3SD from the mean-Action  
Reject batch and investigate problem before repeating analyses.
- Seven consecutive values show a rising tendency

- Seven consecutive values show a falling tendency
- Seven consecutive values lie on one side of the mean

- **Westgard Multirule Chart and rules**

Introduce two control specimens into each analytical run one for each of the two concentrations when two different materials have been selected.

Plot the chart by marking the mean , +2SD, - 2SD and + 3SD, - 3SD values.

If both control results are within 2SD from their target the batch is accepted

If at least one control result is more than 2SD from the target, the remaining rules are evaluated in turn, and the batch is rejected if any one of the rules is satisfied. If none is satisfied the batch is accepted.

The situation should be investigated before the next batch is analyzed

- <sup>1</sup> 2 S - Warning-One result more than 2SD from target
- <sup>1</sup> 3S Action- One result more than 3SD from target
- <sup>2</sup> 2 S Action-Two consecutive results more than 2SD from target in same direction
- <sup>R</sup> 4 S Action-Difference between the control results exceeds 4SD.
- <sup>4</sup> 1 S Action-Four consecutive results more than 1SD from target in the same direction
- <sup>10</sup> x Action – Ten consecutive results on the same side of mean

- **Wheeler rules**

A modification of the above using one control in each batch

- <sup>1</sup> 2 S - Warning-One result more than 2SD from target
- <sup>1</sup> 3S - Action- One result more than 3SD from target
- <sup>2/3</sup> 2 S -Action-Two of the last three results more than 3SD from target in same direction
- <sup>4/5</sup> 1 S - Action-Four of the last five results more than 1SD from target in same direction
- <sup>8</sup> x Action- Eight consecutive results on same side of mean

**Other methods of IQC**

- Duplicate sampling
- Yesterday-today sampling for the stable analytes
- Delta checks with previous test to detect errors of specimen identification
- Limit checks to identify results compatible with life which detect transcription errors
- Cusum charts
- Calculation of daily means from patients results
- Exchange of serum between laboratories

### 3.9 External Quality Assessment Scheme

All of the control procedures described so far have focused on monitoring a single laboratory which constitute internal quality control programmes. The comparison of the performance of different laboratories is known as the external quality assessment. The two are complementary activities, internal QC being necessary for the daily monitoring of the precision and accuracy of the analytical method, and external quality assessment being important for maintaining the long term accuracy of the analytical methods.

A laboratory reputed to have good quality control practices of a country participate in an International External Quality Assessment Scheme. This laboratory in-turn initiate External Quality Assessment Scheme(EQAS) at the national level. In a similar fashion EQAS on a regional basis can be started in a large sized country. The underlying principles, methodology and objectives remain same in all these schemes.

The main objectives of EQAs include evaluation of Internal quality control programme of the participating laboratory, establishing inter- laboratory comparability, influencing the reliability of future testing and ensuring the credibility of the laboratory. It stimulates improvements in performance, helps in the identification of common errors. All these eventually aid in accreditation of the laboratory.

The important points to be considered in the organization of EQAS include, decision about frequency of distribution of the panel which in turn depends on the local factors. Too few distributions will not yield desired results and too many will place a strain on the resources. The participating laboratories should treat the QA samples in the same way as the clinical samples, tested by the same personnel who do it in routine set up using the same reagents. QA samples should be transported to the participating laboratories along with the instructions for handing the specimens and a set of format to report the results. The samples should be processed within a stipulated time by the participating laboratories and reported back to the organizing laboratory. The results should be appropriately documented, analysed by a scoring system (Variance Index Score) to determine the present, past and over all performance of the laboratory.

Voluntary participation in an EQAS is the key to success. An EQAS organizing laboratory can run the programme successfully by winning the confidence of the participating laboratories through maintaining confidentiality of the individual reports. However EQAS should not be used for policing or licencing laboratories.

### 3.10 Internal Audit , Proficiency testing and Accreditation

Internal audit is the process of critical review of all the laboratory activities that affect the quality. These could be first, second or third party audits. First part audit is performed by the staff of laboratories themselves to inspect their own system. Another department of the same institute does second part audit while third party audit is done by an outside agency. It is important to understand that in all the audits it is the quality and not the staff that is audited.

Proficiency testing is the process in which simulated patient specimens made from a common pool are analysed by laboratories: the results of this procedure are evaluated to determine the quality of the laboratories' performance. The currently accepted programme to be totally " successful " a laboratory must produce correct results on four out of five specimens for each of the analytes and have an overall score of at least 80% for three consecutive challenges.

Accreditation is a set of procedures by which an authorized body gives formal recognition that a laboratory is competent to carry out certain tests. The elements of accreditation include an accreditation board that has developed well – defined set of standards for accreditation. The board has its own inspectors /assessors for undertaking process of accreditation.