TRAINING MODULE ON QUALITY CONTROL

LABS FOR LIFE PROJECT

Volume 1

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INTRODUCTION

CHAPTER 1: OVERVIEW

Learning Objectives

At the end of this chapter the learners will understand the following

- Overview on quality control in laboratory
- Difference between internal and external control
- Difference between qualitative and quantitative controls
- Difference between ongoing performance evaluation and evaluation of new methods
- How to use this module

1.1. Quality Controls: Ongoing Performance Evaluation: Overview

The principles of quality management, assurance and control have become the foundation by which clinical laboratories are managed and operated. ISO 15189 in Clause 5.6 elaborates the need for **"Assuring the Quality of Examinations"**.

1.1.1 **Process Control** is an essential element of the quality management, and refers to control of the all activities employed in the pre-examination, examination and post-examination processes in order to ensure accurate and reliable reports. **Sample management** and **quality control** processes are a part of process control. While sample management points to the process control in the pre-analytical phase, Quality control (QC) monitors activities related to the examination (analytic) phase of testing. The goal of quality control is to detect, evaluate, and correct errors due to test system failure, environmental conditions, or operator performance, before patient results are reported.

The Quality Control process includes Internal and External controls.

1.1.2 **Internal Quality Control is the measure of precision,** or how well the measurement system reproduces the same result over time and under varying operating conditions. Internal quality control material is usually run at the beginning of each shift, after an instrument is serviced, when reagent lots are changed, after calibration, whenever patient results seem inappropriate or as per selected QC rules.

Though internal quality control is basically a measure of precision, some additional inputs like a target value and the Total Allowable Error for that parameter; the quality control process will take the lab towards a comprehensive evaluation of ongoing method performance. It is therefore vital that while selecting quality control material it

is important to assure that a program of inter- laboratory comparison is available. It is also important that the laboratory takes the necessary steps towards doing the needful in terms of statistical processes.

1.1.3 **External Quality Assurance (EQA) or Proficiency Testing (PT):** The term external quality assessment (EQA) is used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory. This comparison can be made to the performance of a peer group of laboratories or to the performance of a reference laboratory.

1.1.4 **Mechanisms of Internal Control:** Quality control processes vary, depending on whether the laboratory examinations use methods that produce quantitative, qualitative, or semi-quantitative results. These examinations differ in the following ways.

1.1.4 **(a) Quantitative Examinations** measure the quantity of an analyte present in the sample, and measurements need to be accurate and precise. The measurement produces a numeric value as an end-point, expressed in a particular unit of measurement. For example, the result of blood glucose might be reported as 100 mg/dL.

1.1.4 **(b) Qualitative Examinations** are those that measure the presence or absence of a substance, or evaluate cellular characteristics such as morphology. The results are not expressed in numerical terms, but in qualitative terms such as “positive” or “negative”; “reactive” or “non-reactive”; “normal” or “abnormal”; and “growth” or “no growth”. Examples of qualitative examinations include microscopic examinations, serologic procedures for presence or absence of antigens and antibodies, and many microbiological procedures.

1.1.4 **(c) Semi-Quantitative Examinations** are similar to qualitative examinations, in that the results are not expressed in quantitative terms. The difference is that results of these tests are expressed as an estimate of how much of the measured substance is present. Results might be expressed in terms such as “trace amount”, “moderate amount”, or “1+, 2+, or 3+”. Examples are the commonly used tests such as urine tests using dipsticks, Benedict’s, heat and Acetic acid tests etc. In the case of serologic testing, the result is often expressed as a titer; again involving a number but providing an estimate, rather than an exact amount of the quantity present.

Some microscopic examinations are considered semi-quantitative because results are reported as estimates of the number of cells seen per low power field or high power field. For example, a urine microscopic examination might report 0-5 red blood cells seen per high power field.

So, different QC processes are applied to monitor quantitative, qualitative, and semi-quantitative tests.

1.1.5 **Steps for Implementing and Maintaining a QC Program**

Regardless of the type of examination that is performed, steps for implementing and maintaining a QC program include:

a. establishing written policies and procedures, including corrective actions;
b. training all laboratory staff;
c. assuring complete documentation;
d. reviewing quality control data daily by designated staff to assess validity of the run
e. review of the data at pre-assigned intervals as per the QC protocol by supervisory staff to understand system changes

1.2 Method Evaluation

In addition to Assuring the Quality of Examinations as an ongoing process, ISO 15189, in Clause 5.5 mandates the need for evaluation or verification of methods both before it is used for patient reporting and periodically, at defined intervals. Methods are generally validated by the manufacturer. However, the claims need to be verified before patient reporting is done by the method. The claims of precision, accuracy, linearity, biological reference ranges need to be verified by the lab. It will also be in the lab's interest to pre-verify suitability of the method, before purchase as part of the URS. An FDA approved method just means that the claimed performance specification has been verified. It does not necessarily mean that the method performance will be acceptable. The onus is on the lab to understand this and pre-verify the suitability of the method and fitness for purpose.

Validation - confirmation through the provision of objective evidence that requirements for a specific intended use or application have been fulfilled (ISO 9000).

Verification - confirmation through the provision of objective evidence that specified requirements have been fulfilled (ISO 9000).

1.3 Objectives of the Module

The module is written keeping in mind the needs of Indian public health labs, to introduce the concept of quality and to enable the implementation of a robust quality control system. Assuring the quality of examinations is a requirement as per ISO 15189: 2012. Both internal quality controls and external quality controls (Proficiency Testing) are discussed. Internal controls are discussed with reference to daily monitoring using LJ charts as well as evaluation of ongoing method performance using sigma metrics. Proficiency Testing (EQA) will include the options of PT programs for different disciplines, interpretation of results and remedial actions. In addition, Method Evaluation (ME) is included as it is also a requirement of the ISO 15189:2012.

1.4 Target Audience

The target audience for this manual is the laboratory professionals, doctors and technicians who do clinical laboratory testing.

1.5 Method

Regional trainings will be conducted for all institutions served by Labs for Life. Activity sheets, handouts, PPTs than can be used for onward training are developed and distributed. In addition, Labs for Life website has a QC toolkit for all the statistical activities described in this manual. A digitalized version of this module will also be available soon on the Labs for Life website.

1.6 How to Use the Module

This module is published in 2 volumes. In the first volume the statistical methods employed in lab - Quality Controls; Internal and External; Method Evaluation and Continual Improvement - are described. This as per the requirements of ISO 15189:2012, Clauses 5.6, 5.5 and 4.12. In Volume 2 the Semi quantitative and qualitative control mechanisms used In Microbiology, Hematology, Clinical Pathology, Histopathology and Cytology labs are explained.

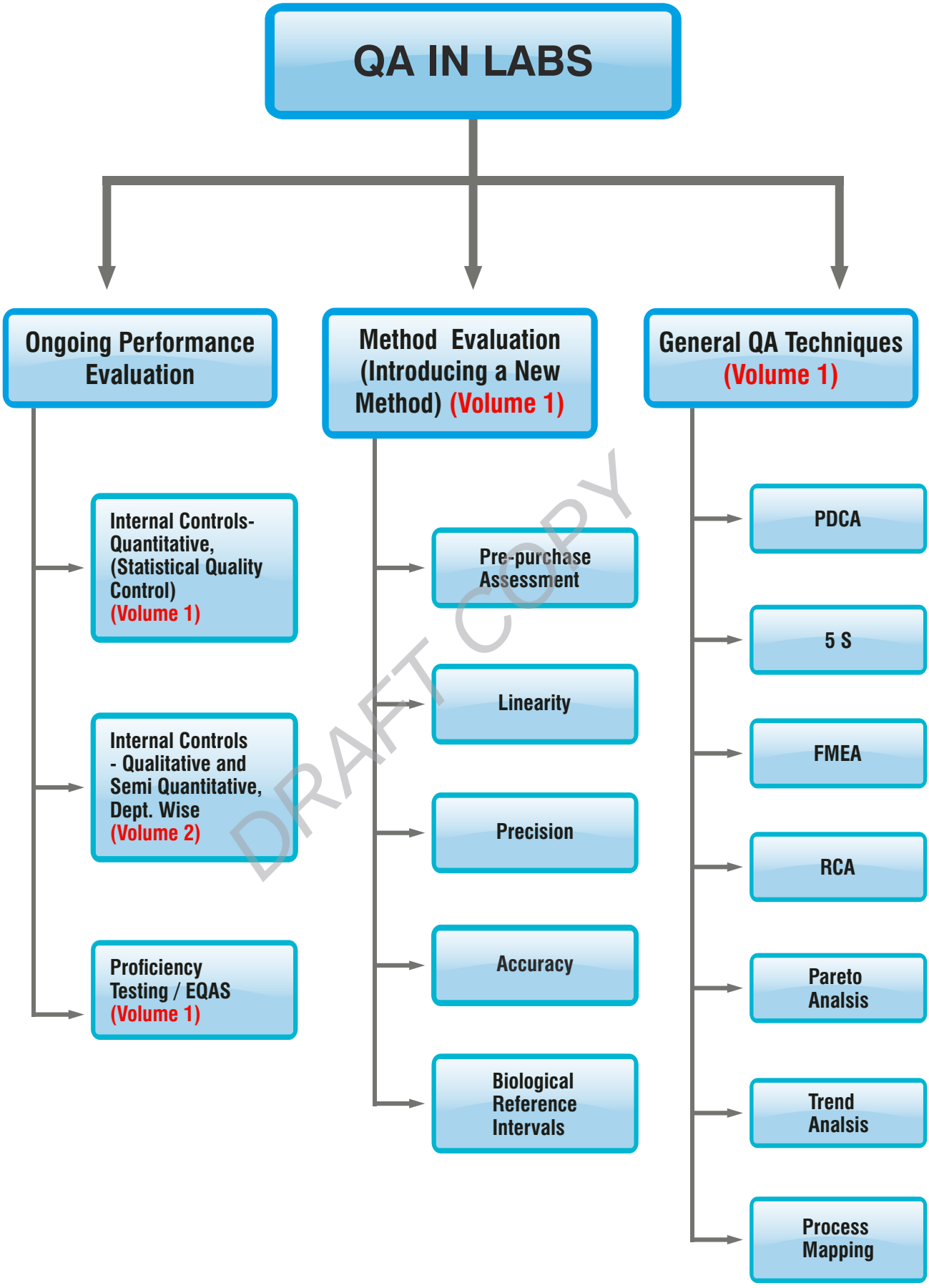


Figure 1: How to use the module

VOLUME 1: STATISTICAL METHODS USED IN A LAB

Part 1: ON-GOING PERFORMANCE EVALUATION

Chapter 1: Introduction

This chapter describes the general overview of Quality Control in a lab, outlining the mechanism for on-going performance evaluation using internal and external controls of different kinds. It also outlines the need for Method Evaluation of any new test or equipment introduced to the lab.

Chapter 2: Internal Controls : Quantitative

It outlines best practices in selecting control materials. The basic concepts in SQCs are then explained in detail. How the characteristic feature - the Gaussian distribution of values - seen in repeated examination of appropriately preserved biological material is made use of for performance evaluation of methods and machines is explained. Every section is supported by worksheets to reinforce the concept explained. The use of Internal QCs for plotting Levey Jennings graph to assess the precision as well as shift in accuracy is detailed. The concept of more advanced interpretations of IQC in terms of Total Error and Sigma metrics is also explained with details of multi-rule selections in the case of poorly performing parameters. The concept of Uncertainty of Measurement as a tool for reporting the confidence levels of a lab's performance is explained. Using a lot of QC as per new guidelines is described. Some specific control mechanisms employed in certain equipments, such as radar graphs, Bull's Algorithm are also explained. The concept of harmonization of equipment as an indicator of comparability of methods has been described.

Chapter 3: Proficiency Testing/ External Quality Assurance

This chapter describes the mechanisms of testing the proficiency of your lab. It outlines the ISO requirements therein and under this scope describes how several mechanisms of proficiency testing can be interpreted. Details of scoring systems and judging acceptance as well as a list of commonly used EQA Schemes in India is given.

Part 2: INTRODUCING A NEW METHOD OR EQUIPMENT

Chapter 4: Method Evaluation

When a new test or equipment is introduced into a lab a mechanism for verifying this is required. A mechanism may be incorporated into the purchase policy of the lab to assess the 'fitness for use' even before an equipment is purchased. These are explained in this chapter.

Part 3: CONTINUAL IMPROVEMENT

Chapter 5: General Concepts in Quality Assurance

ISO 15189 mandates that the lab monitor and assess performance and evolve mechanisms for continual improvement. It also calls for risk assessment and risk management. This chapter outlines a few of these mechanisms with examples.

VOLUME 1: NON-STATISTICAL QUALITY CONTROLS

Part 4: On-going Evaluation of Method Performance: Semi Quantitative and Qualitative Controls

Chapter 6: Internal Control Semi Quantitative and Qualitative Controls: Overview

A general introduction to non-statistical methods of QCs are outlined in this chapter.

Chapter 7: QC in Microbiology and Serology, Quantitative, Semi Quantitative and Qualitative

All aspects of a microbiology lab including bacteriology, parasitology, and mycology are explained. Antibiotic susceptibility testing mechanisms are described. Outlines of serology and molecular diagnostics are also explained in terms of Quality Assurance.

Chapter 8: IQC (Qualitative) in Hematology and Clinical Pathology

This chapter describes a few points to keep in mind, where making blood and bone marrow films are concerned. Some general errors in doing ESR are pointed out. Control mechanisms including pre-analytical and post analytical are enumerated for cavity fluids, urine analysis and semen analysis.

Chapter 9: Quality Assurance in Histopathology and Cytology

The processes that happen in histopathology and cytology labs are several. Each step includes chances of potential error. These should be understood and avoided as part of the quality assurance process. To this end, each step in elaborated with suggestions of how to manage an error free histopathology and cytology lab.

VOLUME 1

PART 1

ONGOING EVALUATION OF METHOD PERFORMANCE

ISO 15189:2012 5.6

ASSURING THE QUALITY OF EXAMINATIONS

CHAPTER 2: INTERNAL CONTROLS: QUANTITATIVE (STATISTICAL QUALITY CONTROLS)

Learning Objectives

At the end of this chapters the learners will be able to answer the following questions:

- How to select, reconstitute, store and use the quality control materials
- The details of quality control material
- Evolution of Quality Control techniques and monitoring mechanism through statistical process like LJ, Total Error and sigma metrics
- How to handle a new lot of quality control
- How to set quality requirements for a lab
- How to plan a QC program in a lab
- Concepts of Uncertainty of Measurement

Quantitative tests measure the quantity of a substance in a sample, yielding a numeric result. For example, the quantitative test for glucose can give a result of 110 mg/dL. Since quantitative tests have numeric values, statistical tests can be applied to the results of quality control material to differentiate between test runs that are "in control" and "out of control". This is done by calculating acceptable limits for control material.

As a part of the quality management system, the laboratory must establish a quality control program for all quantitative tests. Evaluating each test run in this way allows the laboratory to determine if patient results are accurate and reliable.

2.1. Internal Controls: Overview

2.1 (a) Characteristics of Control Materials

It is critical to select the appropriate control materials. Some important characteristics to consider when making the selection.

- Controls must be appropriate for the targeted diagnostic test–the substance being measured in the test must be present in the control in a measurable form.
- The amount of the analyte present in the controls should be close to the medical decision points of the test; this means that controls should check both low values and high values.
- Controls should have the same matrix as patient samples; this usually means that the controls are serum-based, but they may also be based on plasma, urine, or other materials.
- Because it is more efficient to have controls that last for some months, it is best to obtain control materials in large quantity.
- The shelf life and open vial stability of the control should be good, with minimal vial to vial variability and should be stable for long periods of time.

- Should be simple to use.
- Liquid controls are more convenient than lyophilized controls because they do not have to be reconstituted minimizing pipetting error.
- The assayed control providers should provide a robust Inter Laboratory Comparison Program.

2.1 (b) Types and Sources of Control Material

- Control materials are available in a variety of forms. They may be frozen, freeze-dried, or chemically preserved. The freeze dried or lyophilized materials must be reconstituted, requiring great care in pipetting in order to assure the correct concentration of the analyte.

ASSAYED	Target value predetermined Verify and use
UNASSAYED	Target value not predetermined Full assay required before using
"IN-HOUSE"	In-house pooled sera Full assay, validation

Figure 2: Difference between Assayed, Un-assayed and In-House Control

- Control materials may be purchased, obtained from a central or reference laboratory, or made in-house by pooling sera from different patients.
- Purchased controls may be either assayed or un-assayed.
- Assayed controls have a pre-determined target value, established by the manufacturer. When using assayed controls the laboratory must verify the value using its own methods. Assayed controls are more expensive to purchase than un-assayed controls.
- Assayed controls are more expensive to purchase than un-assayed controls.
- When using either un-assayed or "in-house" or homemade controls, the laboratory must establish the target value of the analyte.
- The use of in-house controls requires resources to perform validation and testing steps. An advantage is that the laboratory can produce very large volumes with exact specification.

2.1 (c) Availability

Controls are usually available in 'high', 'normal', and 'low' ranges.

Shown in the graphic are normal, abnormal high and low, and critical high and low ranges.

For some assays, it may be important to include controls with values near the low end of detection.

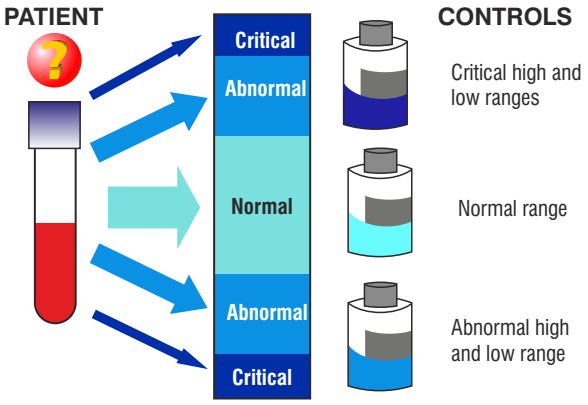


Figure 3: Different Levels of Controls to monitor Clinical Decision Levels

2.1 (d) Preparing and Storing Control Material

Every new QC should be indexed as per the lab's document control protocol. Every time a new QC lot is used the QC literature should be indexed, control stamped and filed. The dates of manufacture, expiry and reconstitution should be noted down. The old QC insert should be stamped obsoleted. Acceptance testing of QC material is discussed along with Lot Verification.

2.1 (e) Reconstitution Procedure

When preparing and storing quality control materials it is important to carefully adhere to the manufacturer's instructions for reconstituting and for storage. Reconstitution of QCs, whether internal or external, should be done with utmost caution. Use a calibrated pipette to deliver the exact amount of required diluent to lyophilized controls that are reconstituted. It would be ideal to use a separate pipette for reconstitution. Carefully including every particle of the lyophilized material stuck to the bottom of the cap is vital. Reconstitution errors can masquerade as system errors and lead to unnecessary corrective actions. Replace the stopper and allow to stand for the time specified, swirling occasionally. Before sampling, gently swirl the vial several times to ensure homogeneity.

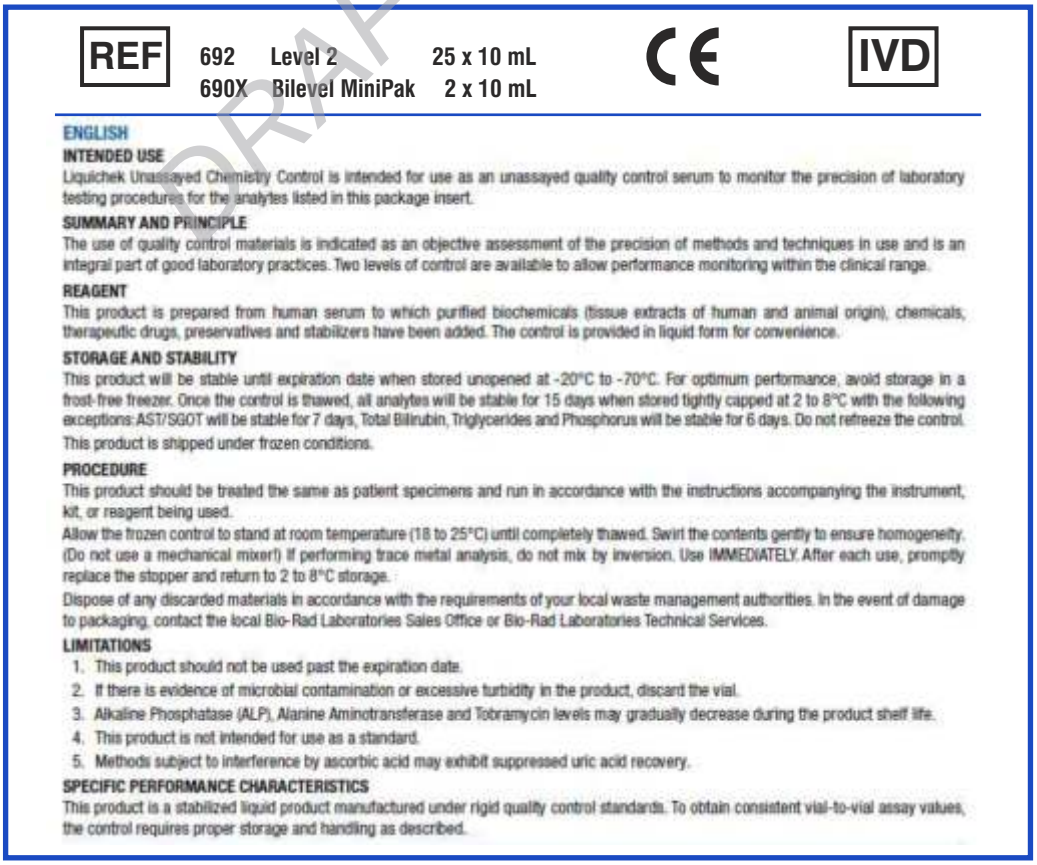
2.1 (f) Storage and Stability

The instructions of the manufacturer should be followed for storage of both unopened and opened vials. For in-house controls, protocols of storage must be done using validated procedures. Divide into aliquots of appropriate volumes and store at -10 °C to -20°C or as specified by the manufacturer. Care should be taken that the aliquots made will not be used beyond the date of expiry. The frozen samples should be thawed at room temperature before being used for assays. Do not thaw and re-freeze control material. Monitor and maintain freezer temperatures to avoid degradation of the analyte in any frozen control material.

In the case of liquid controls, understand the storage requirements, the need for aliquoting.

In the case of hematology controls, there the guidelines on the maximum number of cap opening or piercing should be understood and followed.

If in-house control material is used, freeze aliquots and place in the freezer so that a small amount can be thawed and used daily. An example of a QC insert is given below:



REF 692 Level 2 25 x 10 mL
690X Bilevel MiniPak 2 x 10 mL
CE IVD

ENGLISH

INTENDED USE
Liquichek Unassayed Chemistry Control is intended for use as an unassayed quality control serum to monitor the precision of laboratory testing procedures for the analytes listed in this package insert.

SUMMARY AND PRINCIPLE
The use of quality control materials is indicated as an objective assessment of the precision of methods and techniques in use and is an integral part of good laboratory practices. Two levels of control are available to allow performance monitoring within the clinical range.

REAGENT
This product is prepared from human serum to which purified biochemicals (tissue extracts of human and animal origin), chemicals, therapeutic drugs, preservatives and stabilizers have been added. The control is provided in liquid form for convenience.

STORAGE AND STABILITY
This product will be stable until expiration date when stored unopened at -20°C to -70°C. For optimum performance, avoid storage in a frost-free freezer. Once the control is thawed, all analytes will be stable for 15 days when stored tightly capped at 2 to 8°C with the following exceptions: AST/SGOT will be stable for 7 days, Total Bilirubin, Triglycerides and Phosphorus will be stable for 6 days. Do not refreeze the control.
This product is shipped under frozen conditions.

PROCEDURE
This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used.
Allow the frozen control to stand at room temperature (18 to 25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer!) If performing trace metal analysis, do not mix by inversion. Use IMMEDIATELY. After each use, promptly replace the stopper and return to 2 to 8°C storage.
Dispose of any discarded materials in accordance with the requirements of your local waste management authorities. In the event of damage to packaging, contact the local Bio-Rad Laboratories Sales Office or Bio-Rad Laboratories Technical Services.

LIMITATIONS
1. This product should not be used past the expiration date.
2. If there is evidence of microbial contamination or excessive turbidity in the product, discard the vial.
3. Alkaline Phosphatase (ALP), Alanine Aminotransferase and Tobramycin levels may gradually decrease during the product shelf life.
4. This product is not intended for use as a standard.
5. Methods subject to interference by ascorbic acid may exhibit suppressed uric acid recovery.

SPECIFIC PERFORMANCE CHARACTERISTICS
This product is a stabilized liquid product manufactured under rigid quality control standards. To obtain consistent vial-to-vial assay values, the control requires proper storage and handling as described.

Figure 4: An Example of QC insert

2.1. (g) Purchasing Quality Controls

We expect QC materials to provide information about what is occurring with the measurement procedure. In other words, we expect the performance of the QC materials to mirror the same effects as what is occurring to our patient samples.

To do this, QC materials should:

1) Mimic the matrix and viscosity of the patient samples being tested
   - Matrix—the base from which control materials are prepared in addition to the preservatives added for stability
   - Matrix effect – the influence of the control material's matrix, other than the concentration of the analyte, on the measurement procedure to produce differing results when compared to other methods while still producing consistent results on patient samples
2) Be both physically and chemically sensitive to changes in the measurement procedure as patient samples
3) Contain concentrations of analytes at or near medical decision points
4) Be available in one lot number that is stable for an extended period of time
5) Be available at different concentration levels to assess the measuring range of the method
6) Remain stable before and after opening a vial as indicated by the manufacturer
7) Produce minimal vial-to-vial variability

In addition to the above stated qualities, other considerations that should be kept in mind are:

1) Use of lyophilized (freeze-dried) controls

- Usually less costly per box than liquid.
- Require a special diluent or deionized Type I water.
- Require availability of clean Class 'A' Volumetric pipets and pipetting bulbs.
- Require staff that is capable of
- Accurately pipetting manually Strictly adhering to reconstitution and mixing instructions provided by the manufacturer
- May experience more vial-to-vial variability (increase imprecision) especially if improper handling and reconstitution occurs
- Frequently has a shorter opened vial expiry interval
- May result in discarding unused portion (hidden cost consideration)

2) Use of liquid controls

- Usually more costly per box than lyophilized
- Eliminates many of the handling and reconstitution errors
- Influence of matrix effect may be greater with the method you use
- Frequently has a longer opened vial expiry interval
- May discard less or none of the product if consumed within opened expiry date

3) Frequency of lot number changes

- Performing parallel testing takes time and money (costs of performing testing on QC materials)
- With each QC material lot number change, lose access to summary or cumulative data
- Recommend to purchase a year supply of the same lot number, when possible but taking into consideration the following:
  - Desired expiration date should be specified at time of purchase
  - Storage issues
  - Difficulties encountered with setting up a standing order with the vendor

4) Vendor considerations

- **Availability of an inter-laboratory comparison program**
- Provide troubleshooting support
- Ability to accommodate standing orders
- Ability to sequester specified lot number and automatically ship and bill as outlined in the purchase agreement

2.1. (h) Classification of Control Material

Dependent control material is a quality control material manufactured under the same quality system as the instrument, kit or method it is intended to monitor and whose performance depends on design inputs from the instrument, kit or method manufacturer.

Dependent controls are typically provided by the instrument manufacturer. This type of control material also includes what is referred to as “in kit” controls; those control materials provided as a part of a discrete test kit. Dependent control materials are often manufactured from the same lot of raw material, using the same manufacturing process, and made in the same facility used to manufacture the instrument, kit or method calibrators. At some point, the manufacturing process for controls and calibrators splits.

Independent (Third Party) Control Material is manufactured outside the quality system used to manufacture the instrument, kit or method it is intended to monitor and whose performance is independent of any design inputs from the instrument, kit or method manufacturer.

Quality control material (assayed or un-assayed) is a medical device intended for use in a test system to estimate test precision and detect systematic analytical deviations that may arise from reagent or analytical instrument variation

Semi-dependent control material is manufactured outside the quality system used to manufacture the instrument, kit or method it is intended to monitor but is manufactured on behalf of and with input from the instrument, kit or method manufacturer.

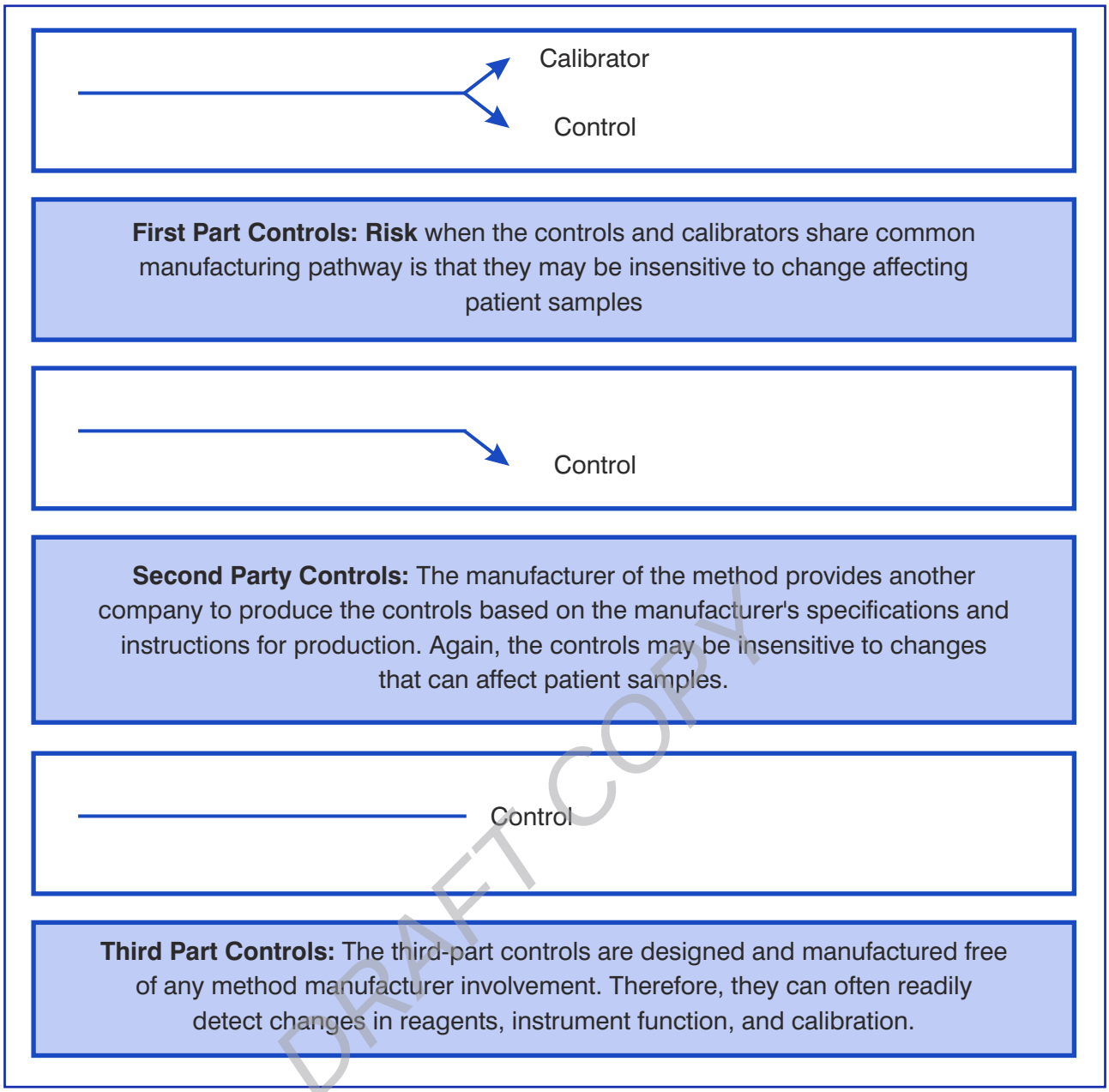


Figure 5: Classification of Control Material

2.2 Quantitative SQCs: Basic Concepts

A characteristic of repeated measurements is that there is a degree of variation. Variation may be due to operator technique, environmental conditions, and the performance characteristics of an instrument. Some variation is normal, even when all of the factors listed above are controlled. The standard deviation gives a measure of the variation.

2.2 (a) Characteristics of repeated measures: Central Tendency

The variability of repeated measurements will be distributed around a central point or location. This characteristic of repeated measurements is known as central tendency.

A few theoretical concepts are important because they are used to establish the normal variability of the test system. Quality control materials are run to quantify the variability and establish a normal range so as to decrease the risk of error

We use statistical terms to describe something about a set of data points. With a specific data set, it is often important to know the values around which the observations tend to cluster. Three measures of the "center" of the data are the mean, the median, and the mode.

Mean ($\bar{x}$) the arithmetic average of results. The mean is the most commonly used measure of central tendency used in laboratory QC)

The mean, also called the arithmetic mean or the average, is the sum of all the data points divided by the number of points. The average is the most common way of calculating central tendency.

Example: For the data set containing 7 numbers {2, 5, 9, 3, 5, 7, and 4}, the mean is calculated as:

$$2+5+9+3+5+7+4 = 35/7 = 5 \text{ is the mean}$$

Some of its characteristics are:

- easy to calculate
- only one exists for any data set
- affected by all observations, and strongly affected by outliers

Median (the central point of the values when they are arranged in numerical sequence.)

The median of a data set is the value of the middle point, when they are arranged in order.

Using the previous data set and arranging from lowest to highest {2, 3, 4, 5, 5, 7, 9}, we can determine the median by crossing off the lowest and highest values, then the next lowest and next highest value. Continue crossing off values from both ends until only one value, the middle value, remains {2, 3, 4, 5, 5, 7, and 9}. For this data set, the median is 5.

If there is an even number of points, average the two middle values.

Example: For the following data set containing 6 numbers, {2, 3, 4, 5, 7, 9}, we can determine the median as follows: 2, 3, 4, 5, 7, 9. for this data set, two numbers, 4 and 5, lie at the center. To determine the median for this data set, we would take the average of 4 and 5 as follows:

$4+5 = 9/2 = 4.5$. The median for this data set is 4.5.

Some characteristics of the median are:

- always exists for a set of data
- unique
- not strongly affected by extreme values
- corresponds to the 50th percentile

Mode (the number that occurs most frequently).

The mode is the value that occurs most frequently in a data set. There can be more than one mode, if there are two or more values that are tied for occurring most frequently. In cases where two numbers occur most frequently, the distribution of data would then be classified as bimodal (having two modes).

For the data set, {2, 5, 9, 3, 5, 7, 4}, all numbers occur only once except the number 5; it occurs twice, or more frequently than the other numbers. Therefore, the mode for this data set is 5.

The properties of the mode are:

- requires no calculation
- not necessarily unique
- very insensitive to extreme values
- may not be close to the center of the distribution

Please refer to exercise no.1

2.2 (b) Normal Distribution: Gaussian is the Key

In probability theory, the normal (or Gaussian) distribution is a very common continuous probability distribution. Normal distributions are important in statistics and are often used in the natural and social sciences to represent real-valued random variables whose distributions are not known. The normal distributions are a very important class of statistical distributions. All normal distributions are symmetric and have bell-shaped density curves with a single peak. To speak specifically of any normal distribution, two quantities have to be specified: the mean, where the peak of the density occurs, and the standard deviation, which indicates the spread or girth of the bell curve.

Many things closely follow a Normal Distribution: heights of people, data points in measurements and blood pressure.

See the distributions below:

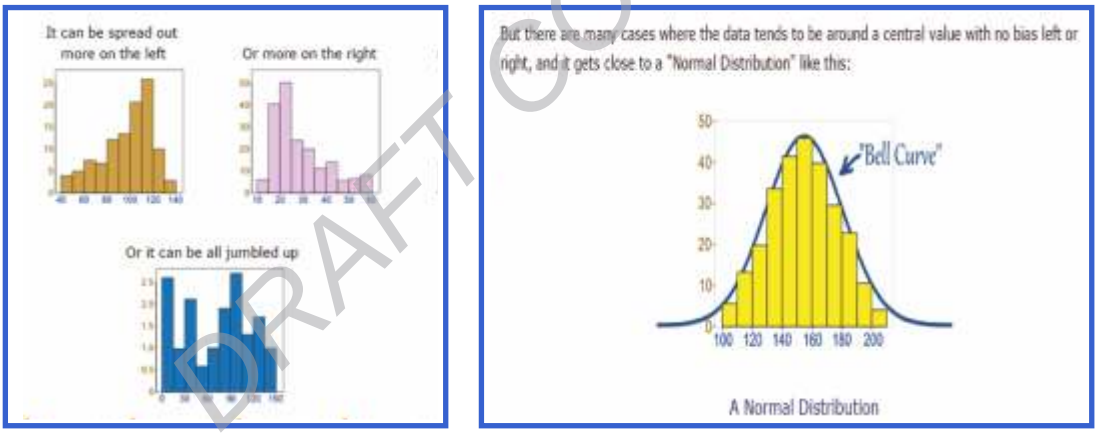


Figure 6: Different Kinds of Distribution

Please refer to exercise no.2

2.2 (c) Some Statistical Notations

Statistical notations are symbols used in mathematical formulas to calculate statistical measures. For this module, the symbols that are important to know are:

$\sum$: the sum of

N: number of data points (results or observations)

$\bar{x}$: the symbol for the mean.

$\bar{\ }$: The square root of the data.

σ : Standard Deviation

2.2 (d) Standard Deviation

Standard Deviation (SD) is a measurement of variation in a set of results. It is the statistic that quantifies how close numerical values (i.e., QC values) are in relation to each other. The term precision is often used interchangeably with standard deviation. Another term, imprecision, is used to express how far apart numerical values are from each other. Standard deviation is calculated for control products from the same data used to calculate the mean. It provides the laboratory an estimate of test consistency at specific concentrations. The repeatability of a test may be consistent (low standard deviation, low imprecision) or inconsistent (high standard deviation, high imprecision). Inconsistent repeatability may be due to the chemistry involved or to a malfunction. If it is a malfunction, the laboratory must correct the problem. It is very useful to the laboratory in analyzing quality control results.

The formula for calculating standard deviation is: $\sigma = \sqrt{[\sum(x - \bar{x})\ ^2 / N-1]}$

The number of independent data points (values) in a data set are represented by "n"

$$s = \sqrt{\frac{\sum(x_n - \bar{x})^2}{n - 1}}$$

Where:

- s = standard deviation
- $\bar{x}$ = mean (average) of the QC values
- $\Sigma(x_n - \bar{x})^2$ = the sum of the squares of differences between individual QC values and the mean
- n = the number of values in the data set

Please refer to exercise no.3

2.2 (e) The 68-95-99.7% Rule

All normal density curves satisfy the following property which is often referred to as the Empirical Rule.

68% of the observations fall within 1 standard deviation of the mean

95% of the observations fall within 2 standard deviations of the mean

99.7% of the observations fall within 3 standard deviations of the mean

Thus, for a normal distribution, almost all values lie within 3 standard deviations of the mean.

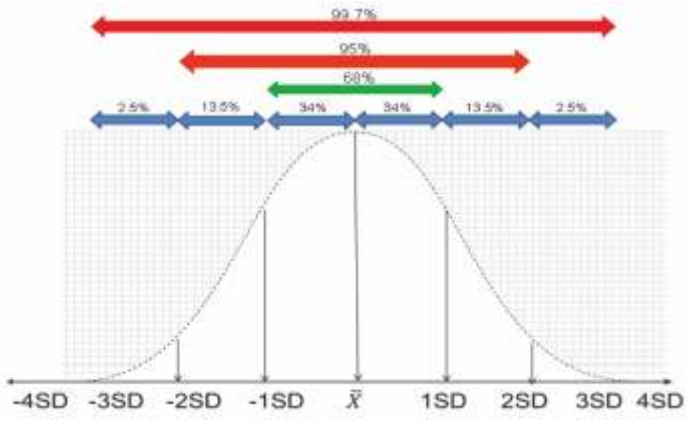


Figure 7: 68-95-99.7 Rule

2.2 (f) Establishing the Value Range for the Control Material

Stable analytical systems will produce the same Gaussian distribution of data when a stable material is run on it, over a period of time. When a system undergoes a change, an unexpected data point will be produced.

One of the most important goals of a quality control program is to differentiate between normal variation and errors.

- **Collecting data**

Once the appropriate control materials are purchased or prepared, the next step is to determine the range of acceptable values for the control material. This will be used to let the laboratory know if the test run is "in control" or if the control values are not reading properly – "out of control".

This is done by assaying the control material repeatedly over time. At least 20 data points must be collected over a 20 to 30 day period. When collecting this data, be sure to include any procedural variation that occurs in the daily runs; for example, if different testing personnel normally do the analysis, all of them should collect part of the data.

Once the data is collected, the laboratory will need to calculate the mean and standard deviation of the results. **Labs For Life QC Tool : Parallel testing of QC**

The purpose of obtaining 20 data points by running the quality control sample is to quantify normal variation, and establish ranges for quality control samples. Use the results of these measurements to establish QC ranges for testing.

If one or two data points appear to be too high or low for the set of data, they should not be included when calculating QC ranges. They are called "outliers".

If there are more than 2 outliers in the 20 data points, there is a problem with the data and it should not be used. Identify and resolve the problem and repeat the data collection.

The measurements are taken when plotted on a graph, it must form a bell-shaped curve as the results vary around the mean as a normal distribution (Gaussian distribution).

The distribution can be seen if **data points are plotted on the x-axis and the frequency with which they occur on the y-axis.**

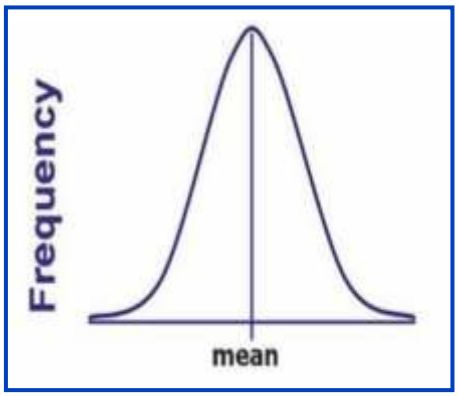


Figure 8: Gaussian distribution plotted alongside time frequency

- **Calculating the Mean, SD, Range**

Also, needing calculation are the Mean and the Standard Deviation as explained above.

Once the mean and the Standard Deviation are understood, the range of acceptability can be assigned and a chart can be developed used to plot the daily control values.

- To calculate 1 SD, add and subtract the value from the mean.
- To calculate 2 SDs, multiply the SD by 2 then add and subtract each result from the mean.
- To calculate 3 SDs, multiply the SD by 3, then add and subtract each result from the mean.

For a mean of 190.5 and an SD of 2, therefore:

- ±1 SD is 188.5 - 192.5
- ±2 SD is 186.5 - 194.5, and
- ± 3 SD is 184.5 - 196.5.

The range of acceptability is ± 3 SD

Once these ranges are established, they can be used to evaluate a test run. For example, if you examine a control with your patients' samples and get a value of 193.5, you know there is a 95.5% chance that it is within 2 SD of the mean.

When an analytical process is within control, approximately 68% of all QC values fall within ±1 standard deviation (1s). Likewise 95.5% of all QC values fall within ±2 standard deviations (2s) of the mean. About 4.5% of all data will be outside the ±2s limits when the analytical process is in control. Approximately 99.7% of all QC values are found to be within ±3 standard deviations (3s) of the mean. As only 0.3%, or 3 out of 1000 points, will fall outside the ±3s limits, any value outside of ±3s is considered to be associated with a significant error condition and patient results should not be reported.

2.2 (g) Graphically Representing Control Ranges: Levey-Jennings Charts

The laboratory needs to document that quality control materials are assayed and that the quality control results have been inspected to assure the quality of the analytical run. This documentation is accomplished by maintaining a QC Log and using the Levey-Jennings chart on a regular basis. The QC Log can be maintained on a computer or on paper. The log should identify the name of the test, the instrument, units, the date the test is performed, the initials of the person performing the test, and the results for each level of control assayed.

The Levey-Jennings charts represent the range graphically for the purpose of daily monitoring.

A Levy-Jennings chart can then be drawn, showing the mean value as well as plus/minus 1, 2, and 3 standard deviations (SD). The mean is shown by drawing a line horizontally in the middle of the graph and the SD are marked off at appropriate intervals and lines drawn horizontally on the graph as shown below.

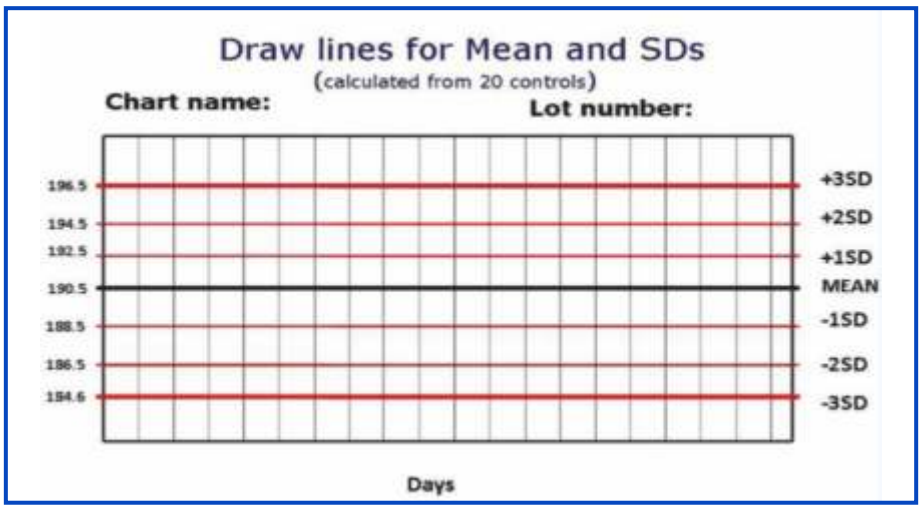


Figure 9: Blank Levy-Jennings chart with defined mean and SD

In order to use the Levey-Jennings chart to record and monitor daily control values, label the x-axis with days, runs, or other interval used to run QC. Label the chart with the name of the test and the lot number of the control being used. On a daily basis, enter values on the chart.

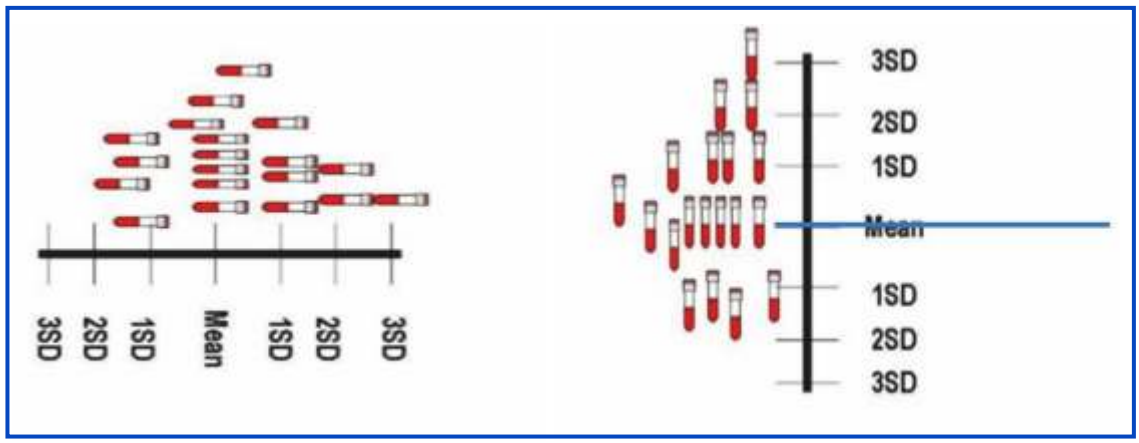


Figure 10: A Gaussian on its side with a frequency, is a LJ Chart

An LJ is basically a Gaussian on its side, separated by time as a frequency. If you look at the figures above and below, this can be understood.

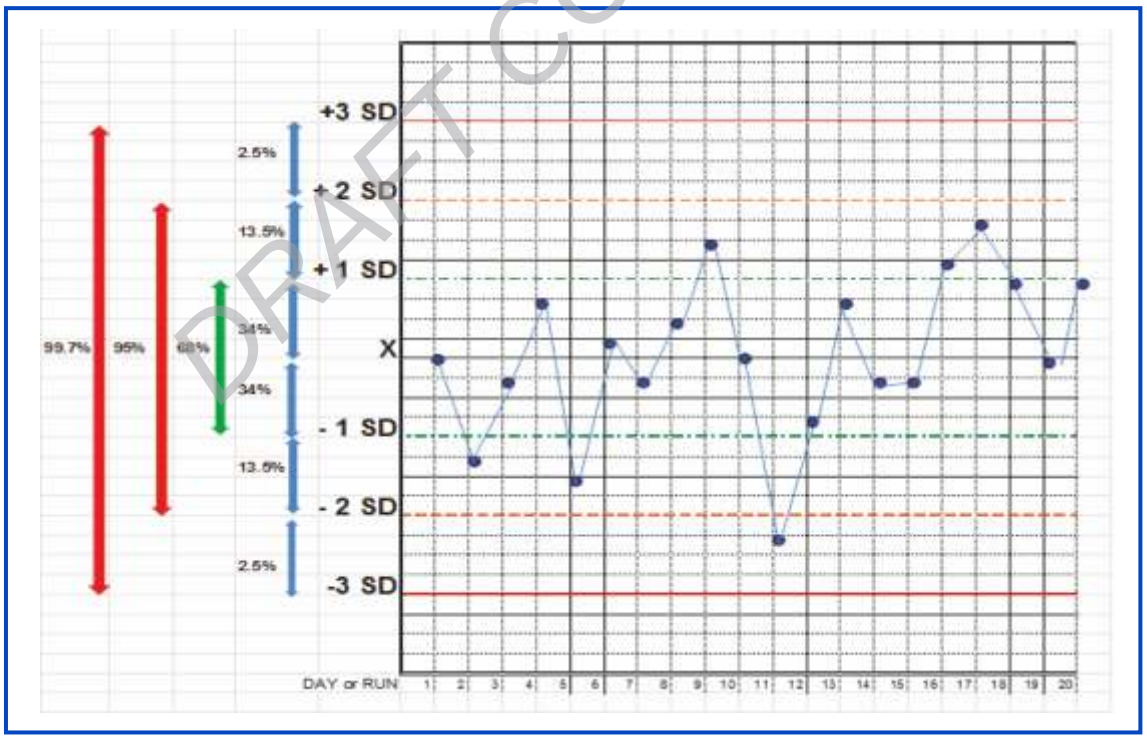


Figure 11: 68-95-99.7 Rule on LJ Chart

Please refer to exercise no.4

2.3 Interpreting Quality Control Data: LJ Charts

2.3 (a) Training your Eyes to Identify Errors and Changes in Pattern

From the above discussion it is evident that the patterns can be easily discerned by eyes once it is graphically represented. This discernment should be both in terms of daily assessments and periodic assessments. A set of rules have been defined that can be used singularly (single rules) or in combination (multi-rules), depending on the performance of the parameter and as protocoled by the lab.

In the following sections, we will examine **the rules, the errors, concepts of accuracy and precision, how to apply the rules to detect errors, how to define the optimum QC protocol for each analyte.**

2.3 (b) The Westgard Rules:

In 1981, Dr. James Westgard of the University of Wisconsin published an article on laboratory quality control that set the basis for evaluating analytical run quality for medical laboratories. The elements of the Westgard system are based on principles of statistical process control used in industry since the 1950s. There are several rules in the Westgard scheme. These rules are used individually or in combination to evaluate the quality of analytical runs.

Westgard devised a shorthand notation for expressing quality control rules. Most of the quality control rules can be expressed as NL where N represents the number of control observations to be evaluated and L represents the statistical limit for evaluating the control observations. Thus 1:3s or 13s represents a control rule that is violated when one control observation exceeds the ±3s control limits.

1. ***1:3s or 1_3s*** *refers to a control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus 3s and the mean minus 3s. A run is rejected when a single control measurement exceeds the mean plus 3s or the mean minus 3s control limit. This rule identifies unacceptable random error or possibly the beginning of a large systematic error. Any QC result outside ±3s violates this rule.*

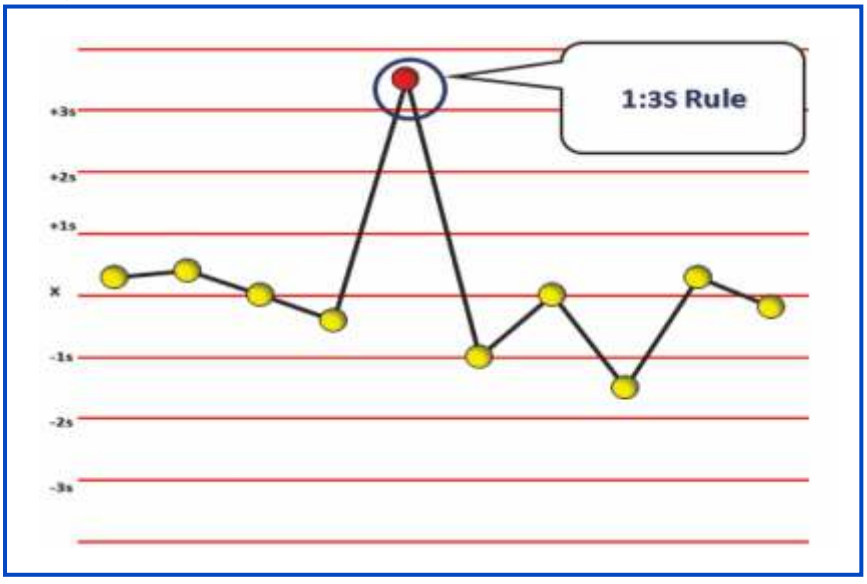


Figure 12: 1:3s or 1_3S denotes a Random Error or a beginning of a Systematic Error

2. ***1:2s or 1_2s*** *refers to the control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus/minus 2s. This is a warning rule that is violated when a single control observation is outside the ±2s limits. Remember that in the absence of added analytical error, about 4.5% of all quality control results will fall between the 2s and 3s limits. This rule merely warns that random error or systematic error may be present in the test system. The relationship between this value and other control results within the current and previous analytical runs must be examined. If no relationship can be found and no source of error can be identified, it must be assumed that a single control value outside the ±2s limits is an acceptable random error. Patient results can be reported.*

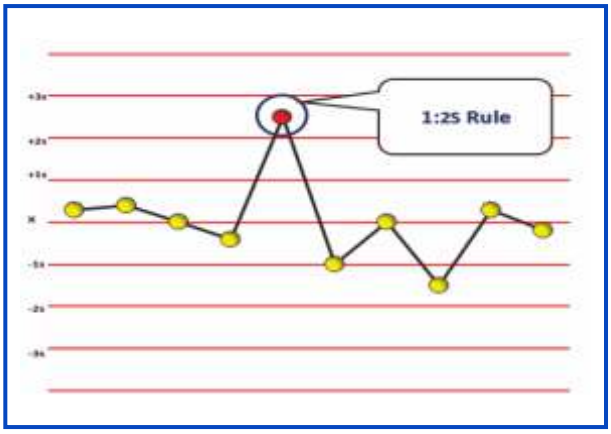


Figure 13: 1:2s denotes a Random Error or a Systematic Error

3. ***2:2s or 2_2s*** *- Two consecutive QC results greater than 2s on the same side of the mean. This rule identifies systematic error only. There are two applications to this rule:* ***within-run*** *(in the 2 levels of QC in the same run) and* ***across runs*** *(In the same QC in 2 consecutive runs). The within-run application affects all control results obtained for the current analytical run. For example, if a normal (Level I) and abnormal (Level II) control are assayed in this run and both levels of control are greater than 2s on the same side of the mean, this run violates the within-run application for systematic error. If however, Level I is -1s and Level II is +2.5s (a violation of the 12s rule), the Level II result from the previous run must be examined. If Level II in the previous run was at +2.0s or greater, then the across run application for systematic error is violated. Violation of the within-run application indicates that systematic error is present and that it affects potentially the* ***entire analytical curve.*** *Violation of the across run application indicates that* ***only a single portion of the analytical curve*** *is affected by the error.*

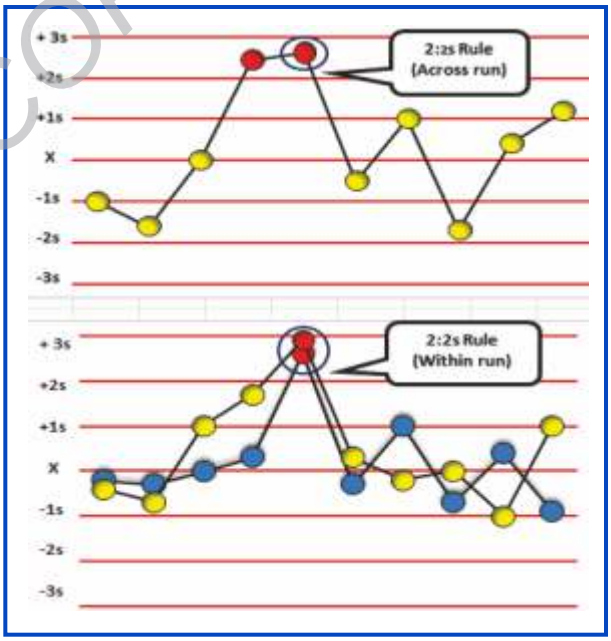


Figure 14: 2:2s denotes a Systematic Error

4. ***2 of 3_2S*** *- when 2 out of 3 control measurements exceed the same mean plus 2s or mean minus 2s control limit;*

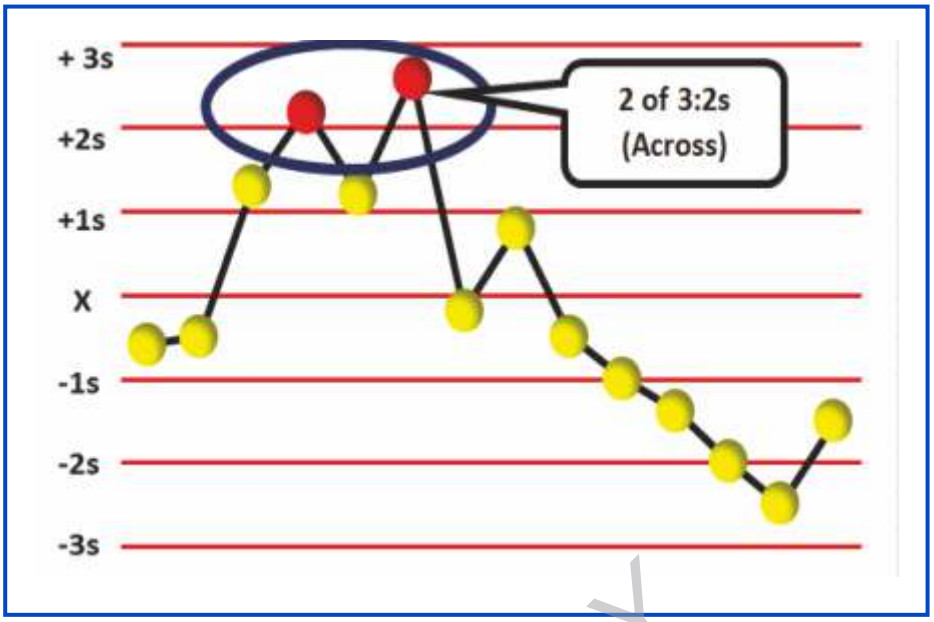


Figure 15: 2 of 3:2s denotes a Systematic Error

5. ***R_4S or R:4S*** *- When 1 control measurement in a group exceeds the mean plus 2s and another exceeds the mean minus 2s. This rule should* ***only be interpreted within-run, not between-run****. This rule identifies* ***random error only****, and is applied* ***only within the current run****. If there is at least a 4s difference between control values within a single run, the rule is violated for random error. For example, assume both Level I and Level II have been assayed within the current run. Level I is +2.8s above the mean and Level II is -1.3s below the mean. The total difference between the two control levels is greater than 4s (e.g. [+2.8s – (-1.3s)] = 4.1s). In the above example, though the Level II has not violated a -2 SD level, together the within run QC violates an R_4S. Some authors validate across run* ***R_4s*** *violations.*

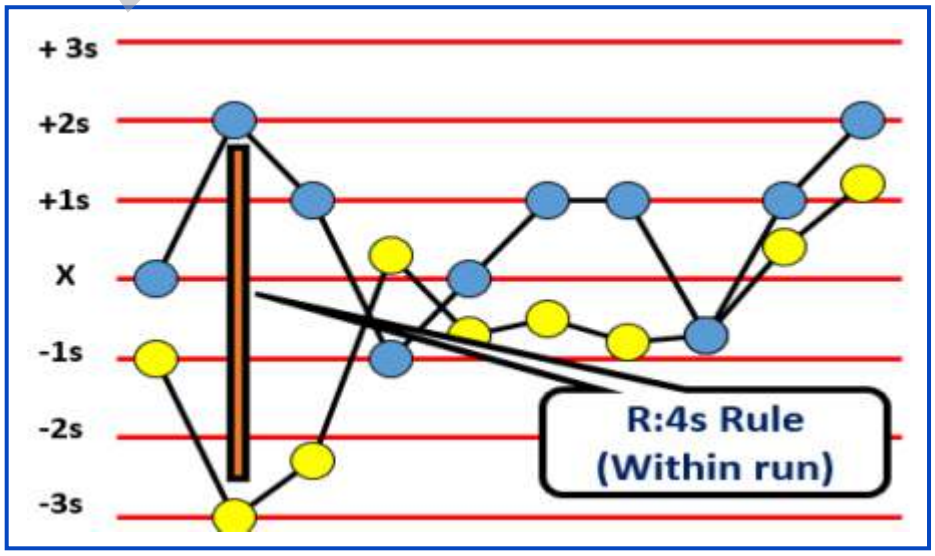


Figure 16: R:4s denotes a Random error

6. ***3,S or 3:1S***- *3 consecutive control measurements exceed the same mean plus 1s or mean minus 1s control limit.*

- *3 consecutive results*
- *Greater than 1s*
- *On the same side of the mean*

These are within control material (e.g. all Level I control results) or across control materials (e.g., Level I, II, and III control results in combination when a tri-level control is used, n=3 or 6). Within control material violations indicate systematic bias in a single area of the method curve while violation of the across control materials application indicates systematic error over a broader concentration.

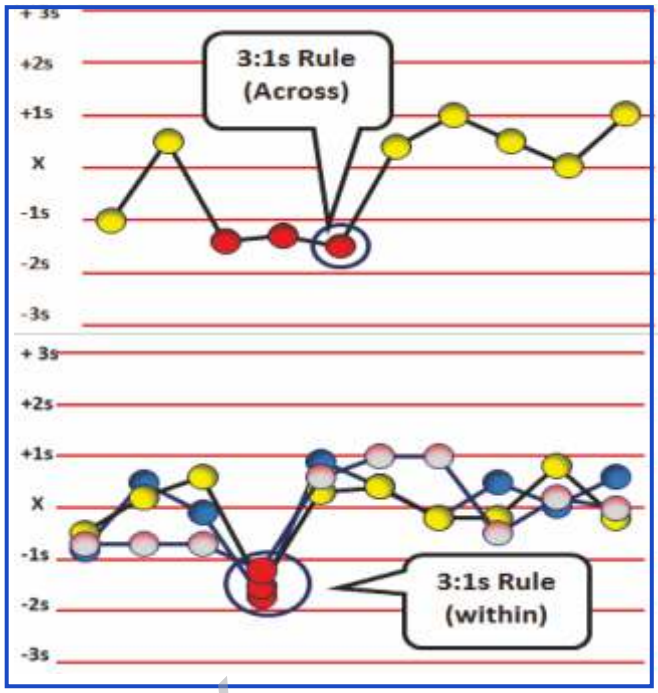


Figure 17: 3:1S Denotes a Systematic Error

7. ***4,S or 4:1S*** - *When 4 consecutive control measurements exceed the same mean plus 1s or the same mean minus 1s control limit.*

- *Four consecutive results*
- *Greater than 1s*
- *On the same side of the mean*

There are two applications to the 3:1S and 4:1S rule. These are within control material (e.g. all Level I control results) or across control materials (when n is 2 or 4). Within control material violations indicate systematic bias in a single area of the method curve while violation of the across control materials application indicates systematic error over a broader concentration.

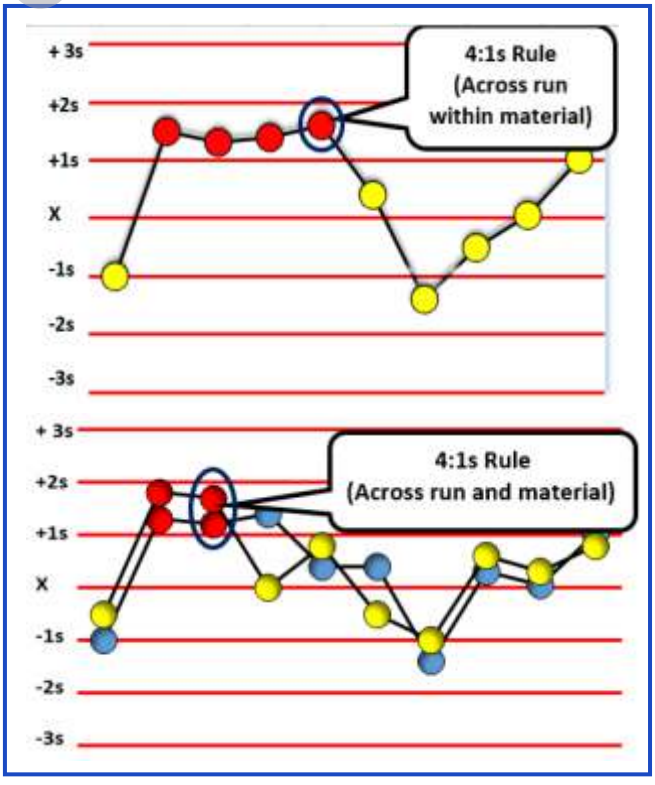


Figure 18: 4:1S denotes a Systematic Error

8. ***6x, 8x,9x, 10x, 12x***

These rules are violated when there are: 6 or 8, or 9, or 10, or 12 control results on the same side of the mean regardless of the specific standard deviation in which they are located.

Each of these rules also has two applications: within control material (e.g., all Level I control results) or across control materials (e.g. Level I, II, and III control results in combination). Within control material violations indicate systematic bias in a single area of the method curve while violation of the across control materials application indicates systematic bias.

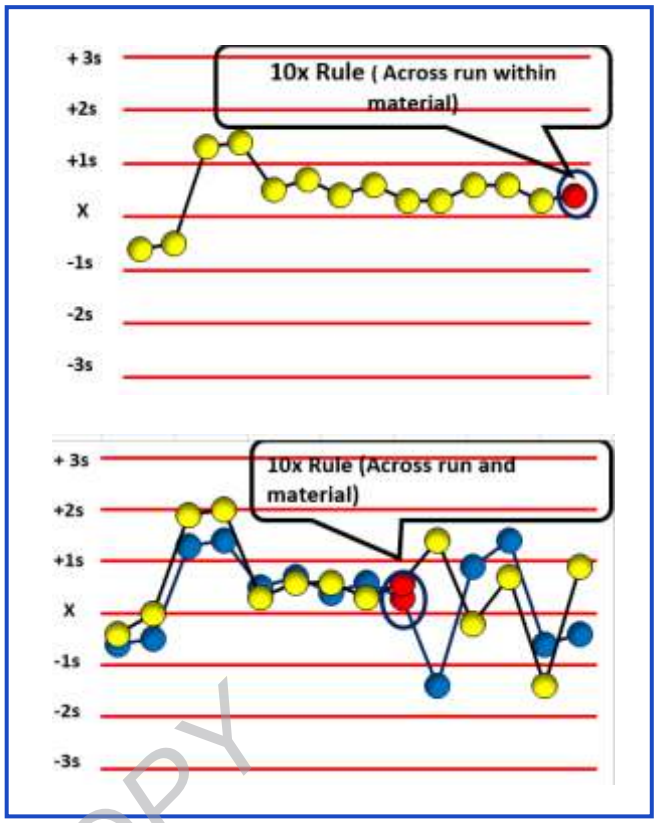


Figure 19: 10x rule denotes Systematic Errors

6x, 8x,9x, 10x, 12x denotes ***Systematic Errors***

9. ***7_T*** *- When seven control measurements trend in the same direction, crossing the mean, i.e., get progressively higher or progressively lower. Applicable across run*

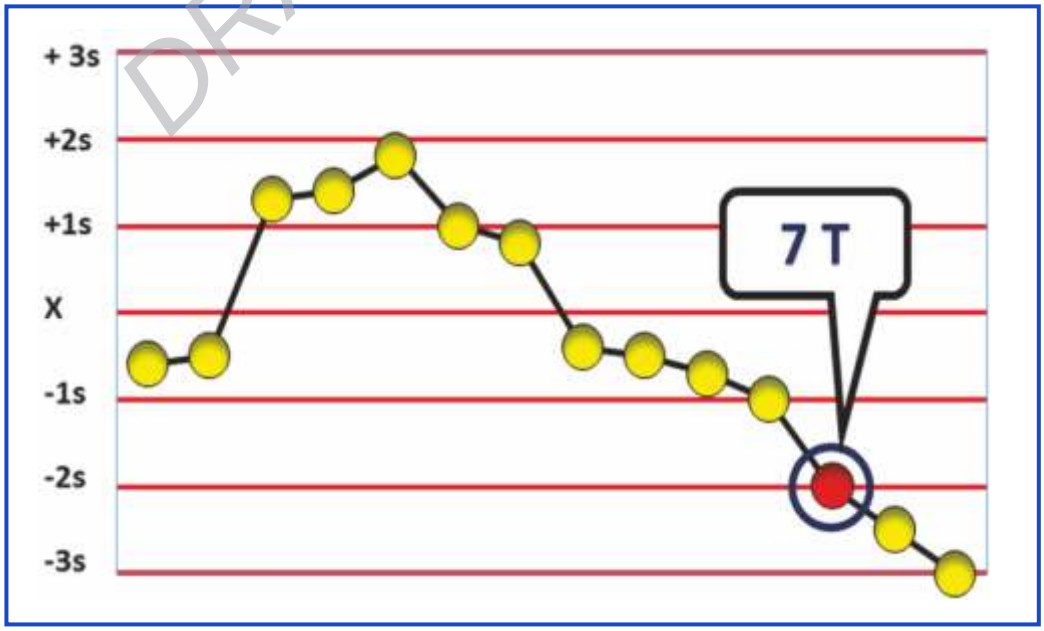


Figure 20:7T denotes a Systematic Error

Please refer to exercise no.5

2.3 (c) Using Only One Level Control

If it is possible to use only one control, choose one with a value that lies within the normal range of the analyte being tested. When evaluating results, accept all runs where the control lies within + 2 SD. Using this system, the correct value will be rejected 4.5% of the time (False Rejects).

2.3 (d) Using the Rules: Single Rule and Multi Rules

Please refer section 2.8 QC Planning for the details of using QC rules in Lab.

2.3 (e) Concepts of Accuracy, Precision and Total Error

If a measurement is repeated many times, the result should be a mean that is very close to the true mean.

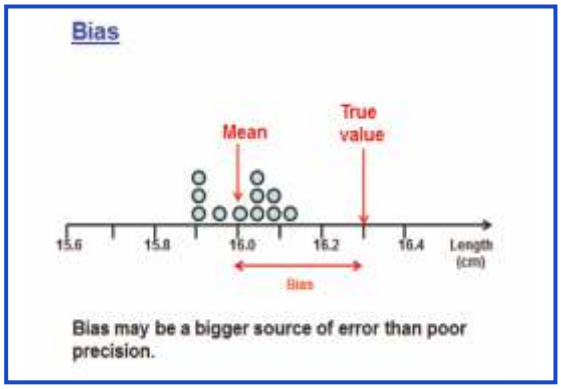


Figure 21: Concept of bias in performance

1) **Accuracy** is the closeness of a measurement to its **target**/ **true** value (explained later). When the mean changes from the true mean, there is measuring system is said to have a **systematic error or bias** Systematic error is evidenced by a change in the mean of the control values.

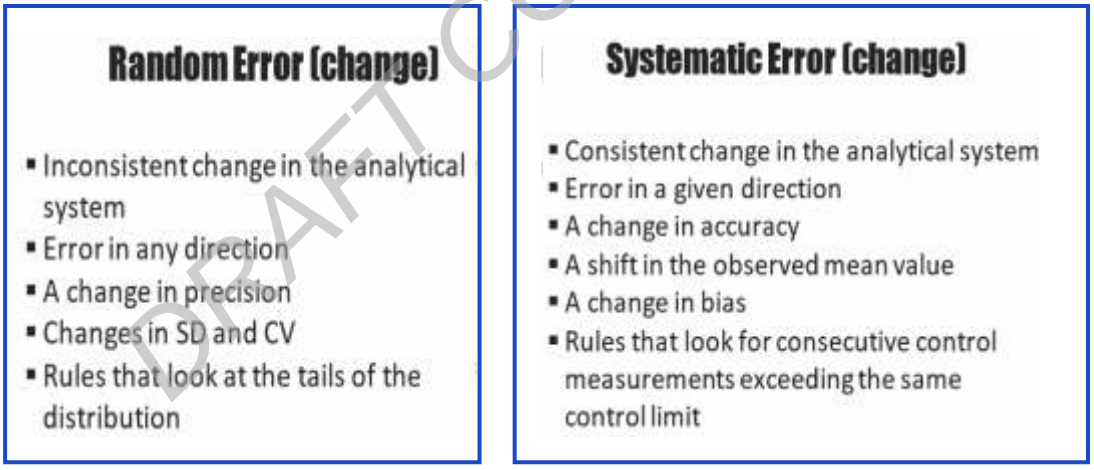


Figure 22: Differences between Random & Systematic Errors

2) The change in the mean may be gradual and demonstrated as a trend in control values or it may be abrupt and demonstrated as a shift in control values. Bias is the difference between true or target value and the obtained value.

Target Value may be obtained from

1) Inter-laboratory comparison programs of the QC manufacturer. Good QC providers give monthly as well as cumulative means. The cumulative means are robust value and will give very good anchoring of the true value
2) Manufacturer assigned mean
3) Long term lab mean provided the QC lot has been running for a considerable duration.

Bias values have direction, it may be Positive or Negative, depending on if the obtained value is higher or lower than the target. It is thus imperative that the absolute value be obtained from the actual bias. Acceptable Bias values are available in BV Charts ***(Annexure no 2:A)***

Bias thus has a value which can be used to eliminate or minimize the offset e.g. by recalibration or by adjusting raw results with a correction factor.

3) **Precision** is the amount of variation in the measurements, a deviation away from an expected result and is computed as **Random Error.** The acceptable (or expected) variations are defined and quantified by standard deviation. There are unacceptable (unexpected) variations when any data point falls outside the expected population of data. The less variation a set of measurements has, the more precise it is. The variation thus is measured in Standard Deviations. In more precise measurements, the width of the Gaussian curve is smaller because the measurements are all closer to the mean. The rule violations will happen in the tails of the Gaussian or upper and lower ends of LJ typically as R_4S or 1_3S violations.

4) **Total Error** is the combined value of both accuracy and precision (Discussed later)

TE= SE + RE, where SE is the Systematic Error (Bias) calculated by subtracting the Obtained Lab Mean from the True (Target) Mean and RE is 1.65 (Z Factor)* SD (or CV)

The reliability of a method is thus judged in terms of accuracy and precision which contributes to the Total Error. A simple way to portray precision and accuracy is to think of a target with a bull's eye.

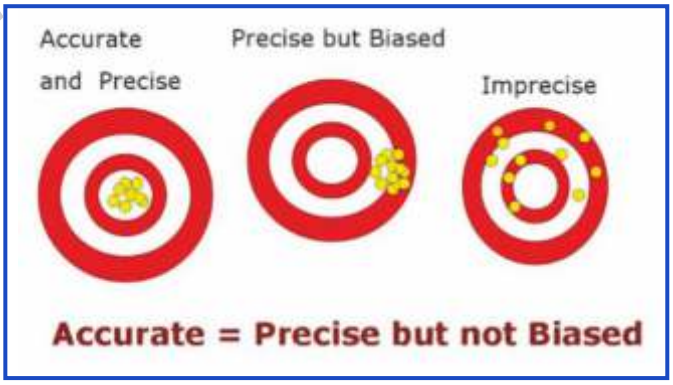


Figure 23: Difference between Accuracy & Precision

The bull's eye represents the accepted reference value which is the true, unbiased value. If a set of data is clustered around the bull's eye, it is accurate. The closer together the hits are, the more precise they are. If most of the hits are in the bull's eye, as in the figure on the left, they are both precise and accurate.

The values in the middle figure are precise but not accurate because they are clustered together but not at the bull's eye. The figure on the right shows a set of hits that are imprecise. Measurements can be precise but not accurate if the values are close together but do not hit the bull's eye. These values are said to be biased. The middle figure demonstrates a set of precise but biased measurements.

The purpose of quality control is to monitor the accuracy and precision of laboratory assays before releasing patient results.

2.3 (f) What Errors can be Detected on the LJ?

Using the LJ graph the following points can be discerned. Look at the examples below.

1. Errors in precision are easily detected. See increasing imprecision towards the second half of the LJ contributing to increased Random Error (Figure 24)
2. A change in accuracy can be observed as an emerging population of data points with a new mean developing indicating a Systematic change (Figure 25)
3. If the Target or True value (explained later) is available, it can be discerned if you are changing for a better or a worse accuracy (explained later)

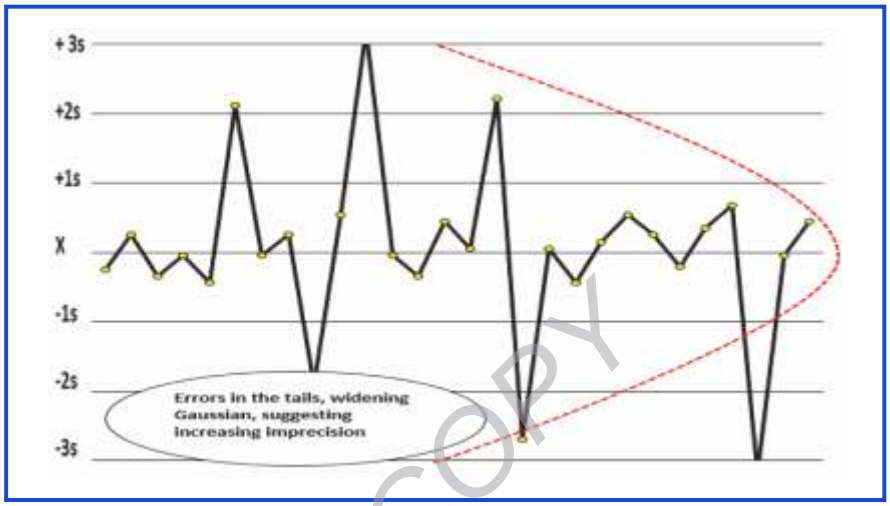


Figure 24: Imprecision

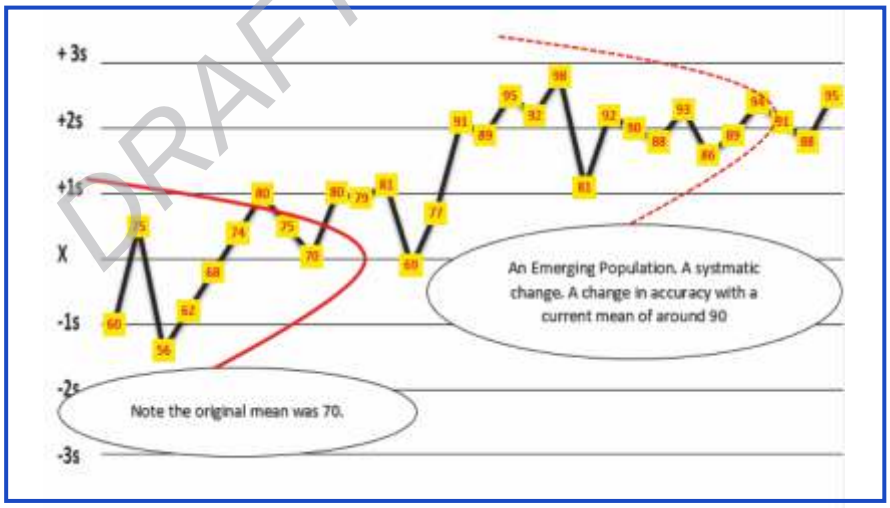
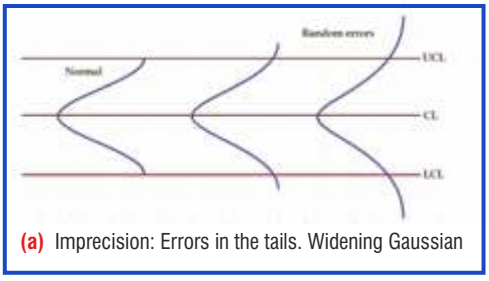
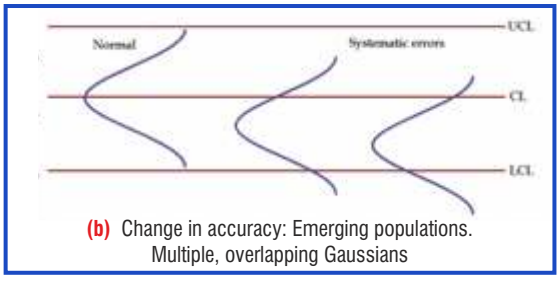


Figure 25: Shifting Accuracy



(a) Imprecision: Errors in the tails. Widening Gaussian



(b) Change in accuracy: Emerging populations. Multiple, overlapping Gaussians

Figure 26: Recap Increasing Imprecision (a) and Shifting Accuracy (b)

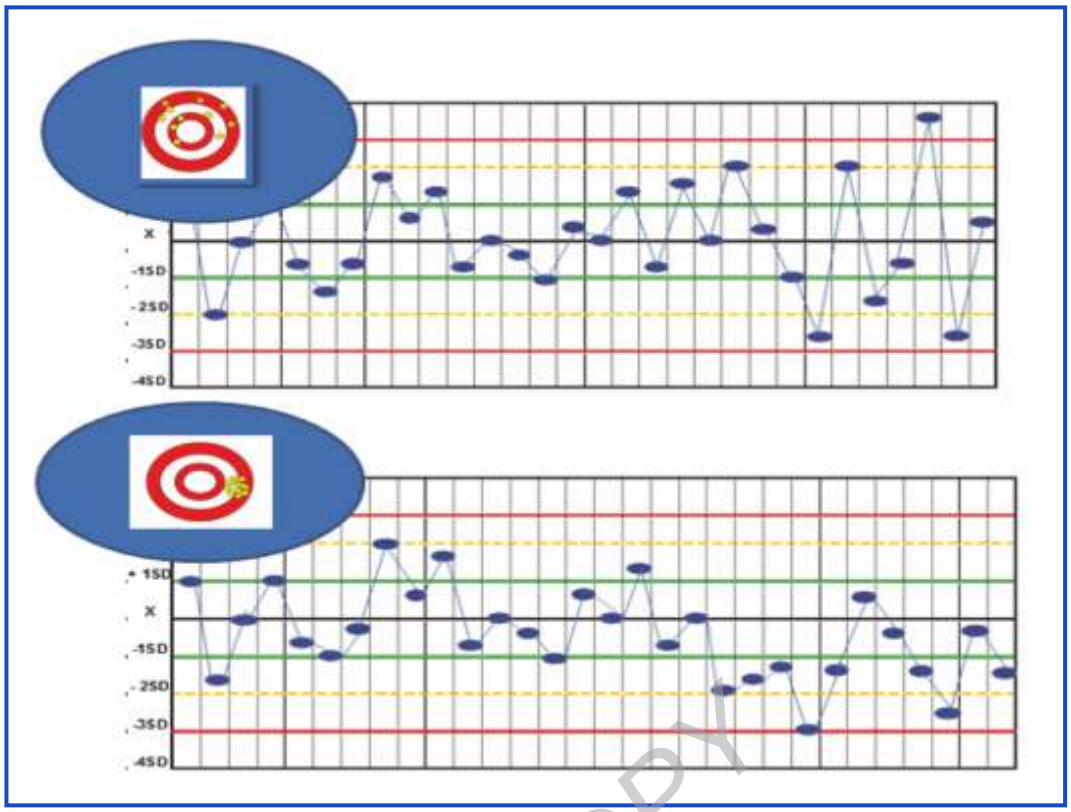


Figure 27: Recap (Real time) Increasing Imprecision (a) and Shifting Accuracy (b)

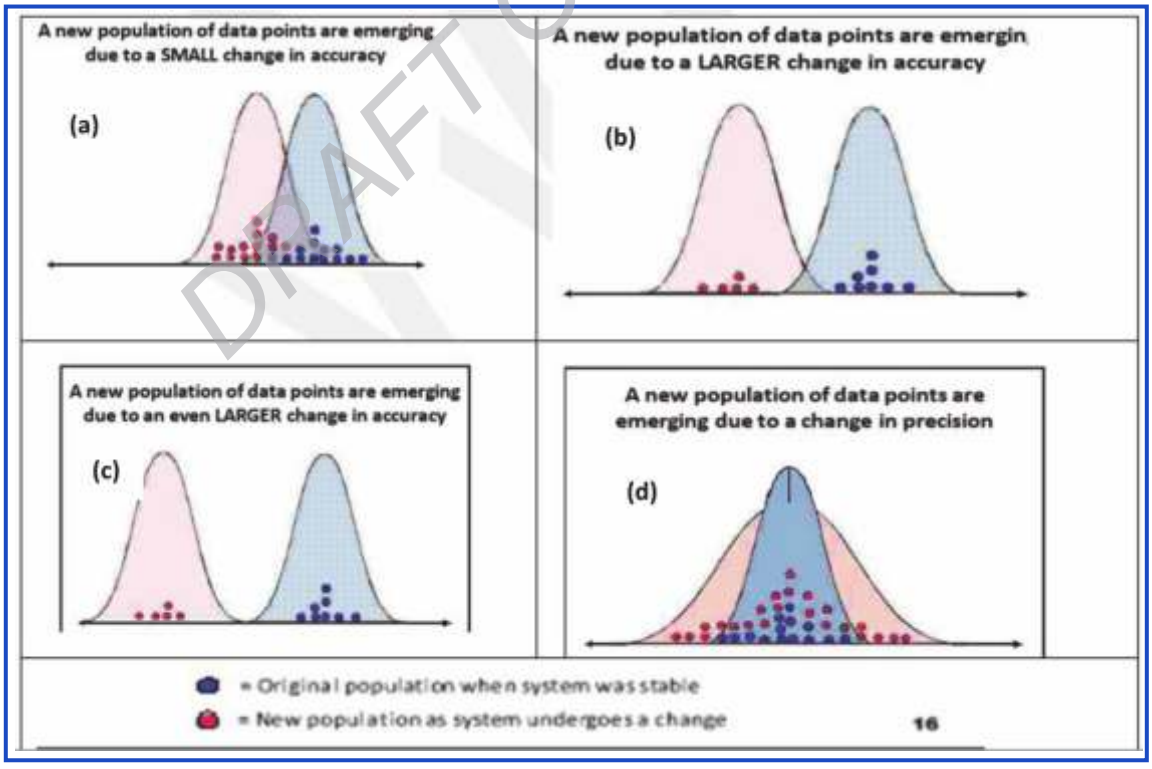
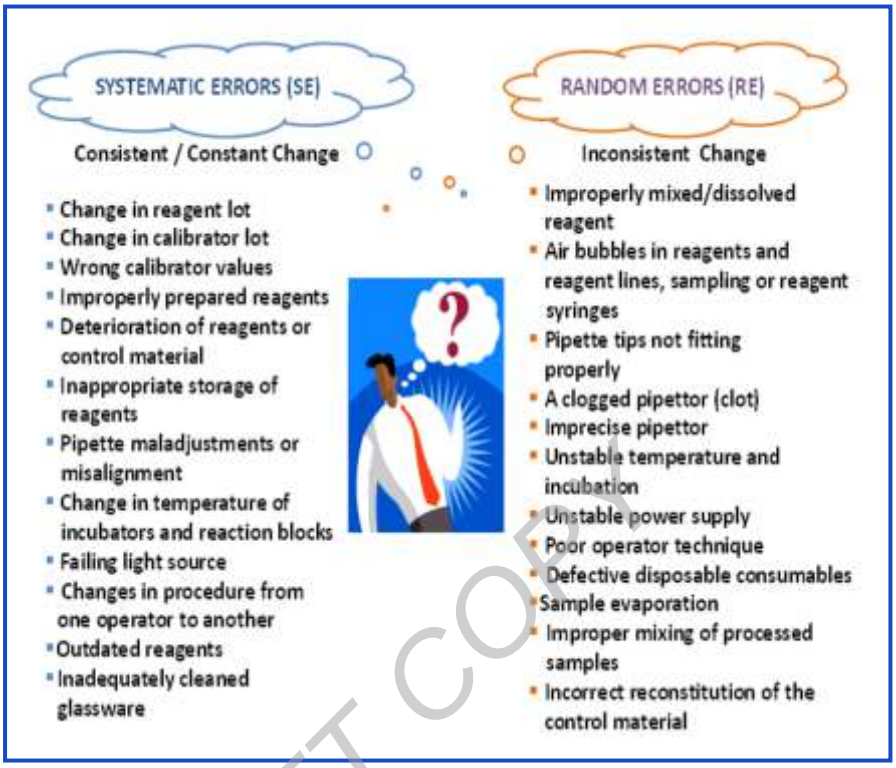


Figure 28: Recap on shifting accuracy and increasing imprecision on a Gaussian: Shifting accuracy (a to c). In figure (a) the two populations are overlapping and is difficult to distinguish an emerging population. In figure (b & c) the shift becomes more pronounced and can be easily understood. In figure (d) increasing imprecision gives rise to populations outside the original Gaussian (Widening Gaussian in pink).

Systematic Errors (SE) are consistent, easy to detect and correct. Random Errors (RE) are inconsistent ad difficult to detect. The quality control program of the lab should be equipped at detecting both kinds of errors to the maximum possible limits.

In the tables below are listed causes of SE and RE and within the SE, the causes of Trends and Shifts



Trend	Shift
A trend indicates a gradual loss of reliability in the test system. Trends are usually subtle. Causes of trending may include:	**Abrupt changes in the control mean are defined as shifts. Shifts in QC data represent a sudden and dramatic positive or negative change in last system performance. Shifts may be caused by:**
• Deterioration of the instrument light source • Gradual accumulating of debris in sample / reagent tubing • Gradual accumulation of debris on electrode surfaces • Aging od reagents • Gradual deterioration of control materials • Gradual deterioration of incubation chamber temperature (enzymes only) • Gradual deterioration of light filter integrity • Gradual deterioration of calibration	• Sudden failure or change in the light • Change in reagent formulation • Change of reagent lot • Major instrument maintenance • Sudden change in incubation temperature (enzymes only) • Change in room temperature or humidity • Failure in the sampling system • Failure in reagent dispense system • Inaccurate calibration / recalibration

As explained earlier, the change in the mean may be gradual and demonstrated as a **trend** in control values or it may be abrupt and demonstrated as a **shift** in control values.

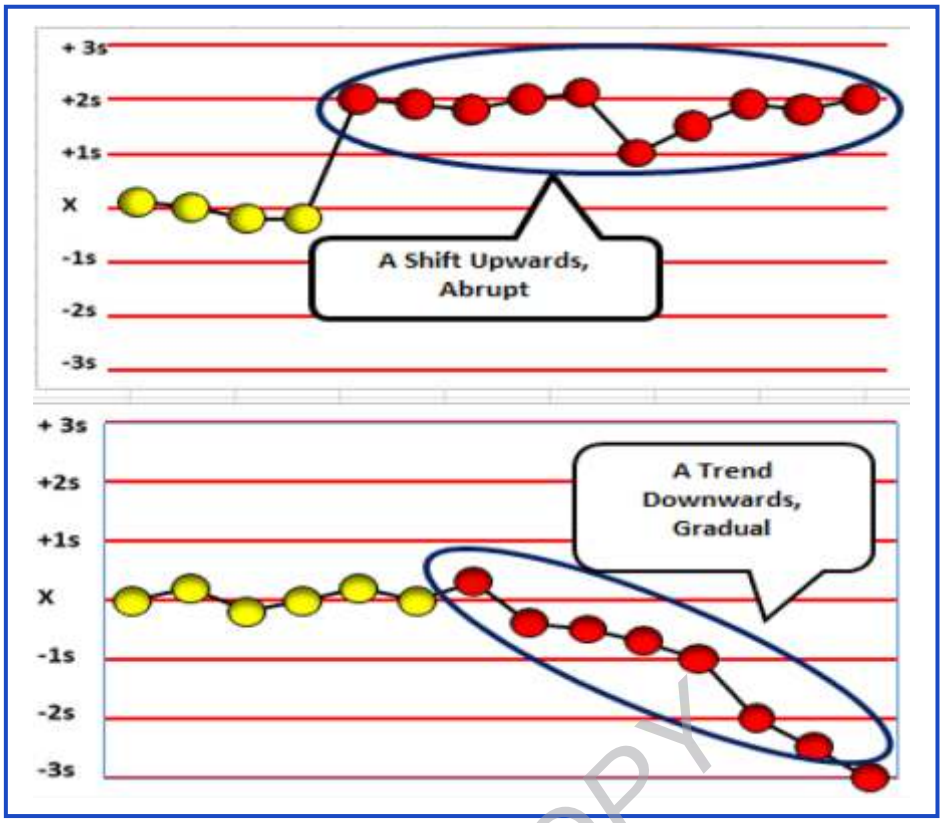


Figure 29: Shifts and Trends

Please refer to exercise no.6

2.3 (g) Other Concepts of Precision

- **Repeatability:** is a condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time. Repeatability may be expressed in terms of multiples of the standard deviation. Within-run/ Intra-serial/Intra-run precision condition are synonyms.
- **Reproducibility:** is precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method in different laboratories, by different operators, using different equipment, in different laboratories, in different locations, or on different days. Reproducibility may be expressed in terms of multiples of the standard deviation. Between Laboratories/ Inter Laboratory/Among Laboratories are synonyms.
- **Intermediate Precision:** Is something between the 2 states, generally meaning with one lab, but with changes of reagent and calibrator lots, operators, operating conditions. All acceptable laboratory variables will be captured if at least 100 measurements are included. The Uncertainly of Measurement (MU) uses intermediate precision as the basis for its calculation.

2.3 (h) Coefficient of Variation

The coefficient of variation (CV) is the standard deviation (SD) expressed as a percentage of the mean.

$$CV\ (\%) = (SD/\ Mean) \times 100$$

The CV is used to monitor precision. When a laboratory changes from one method of analysis to another, the CV is one of the elements that can be used to compare the precision of the methods. As SD is expressed as a percent, it is easier to compare method imprecision in CVs. The Allowable CV limits are defined in several published documents like BV Values and CLIA Proficiency Limits. A suggested guideline is that, for CLIA values, 25% of the values should be used for repeatability and 33% for intermediate precision. In the CLIA chart given below, glucose Proficiency values are given as 10%. So the lab may choose to use 2.5% for repeatability and 3.3% for Intermediate Precision. BV values may be used as such.

Please refer to exercise no.7

2.4 New Lot QC

2.4 (a) Establishing the Value of the Mean for a New Lot of QC Material Labs for Life QC Tool: Parallel testing of QC

The practice of using the Manufacturer stated mean and SD can have a detrimental effect on the patient reporting if the set values are incorrect or inappropriate. Therefore, new lots of a quality control material should be analyzed for each analyte of interest in parallel with the lot of control material in current use Ideally a minimum of at least 20 measurements should be made on separate days when the measurement system is known to be stable, based on QC results from existing lots. If the desired 20 data points from 20 days are not available, provisional values may have to be established from data collected over fewer than 20 days. Possible approaches include making no more than four control measurements per day for five different days. Sampling from at least a few reconstituted vials will include any errors of reconstitution. For liquid stable quality control products, fewer bottles may be required, since such materials are expected to exhibit less vial to vial variation. When an opened bottle of QC material will be used for more than one day, the same bottle should be assayed on several days to allow analyte stability to be reflected in the mean value. Also note that the recommendation for a minimum of 20 days is intended to enable day to day sources of variability in the measurement procedure to be reasonably represented in the mean value.

2.4 (b) Establishing the Value of the Standard Deviation for a New Lot of QC Material

If there is a history of quality control data from an extended period of stable operation of the measurement procedure, the established estimate of the standard deviation can be used with the new lot of control material, as long as the new lot of material has similar target levels for the analyte of interest as for previous lots. The estimate of the standard deviation should be reevaluated periodically.

If there is no history of quality control data, the standard deviation should be estimated, preferably with a minimum of 20 data points from 20 separate days. The analyte stability after opening a control product should also be considered, and the same bottle tested on sequential days to include this source of variability in the estimate of SD. This initial standard deviation value should be replaced with a more robust estimate when data from a longer period of stable operation become available.

Estimates of the standard deviation (and to a lesser extent the mean) from monthly control data are often subject to considerable variation from month to month, due to an insufficient number of measurements (e.g., with 20 measurements, the estimate of the standard deviation might vary up to 30% from the true standard deviation; even with 100 measurements. the estimate may vary by as much as 10%).More representative estimates can be obtained by calculating cumulative values based on control data from

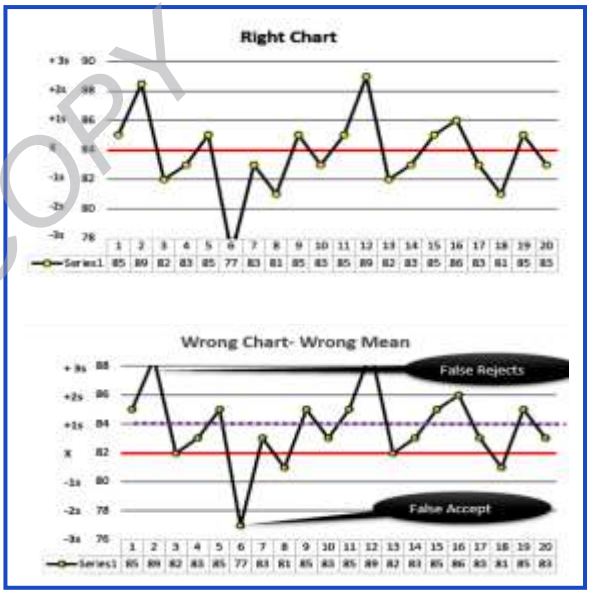
longer periods of time (e.g., combining control data from a consecutive six-month period to provide a cumulative estimate of the standard deviation of the measurement procedure). This cumulative value will provide a more robust representation of the effects of factors such as recalibration, reagent lot change, calibrator lot change, maintenance cycles, and environmental factors including temperature and humidity. Care should be taken to ensure that the method has been stable and the mean has not been drifting consistently lower or consistently higher over the six-month periods being combined, for example due to degradation of the calibrator or control material.

An alternate method is to use the cumulative CV% and the mean obtained to arrive at an attainable and defendable SD.

Please refer to exercise no.2

2.4 (c) Having the Right Control Chart

Quality control procedures should be capable of detecting measurement errors at an appropriately high rate (P ed > 90%) with minimum false accepts (an outlier accepted because the chart did not flag it as an outlier) and minimum false rejections (P fr < 5%)(a valid run rejected because the chart flagged it as an outlier), based on the characteristics of the particular analytical procedure being monitored and the relevant medical requirements for assay quality. To this end, it is important to set the right Mean and Standard Deviation on the chart.



In this graph assume that the SD (2) and mean (84) are correctly assigned. Data point “2” is 1:2s, data point “6” is 1:3s and data point “12”is 1: 2s.

The same data points as in the earlier graph, plotted with the mean of 82. This wrong plotting, results in false rejects at data points “2 & 12” and false accept at data point “6”.

Thus a wrong mean assignment can result in wrong interpretation of LJ graph.

Similarly wrong SD can also result in false accepts and rejects. See figure 30. See the violation of 68-95-99 rule, in both cases, invalidating the SQC concept altogether.

In the upper graph, the SD is too narrow (1). This results in false rejection of many values.

In the lower graph, the SD is too wide (4). This results in false acceptance of many values.

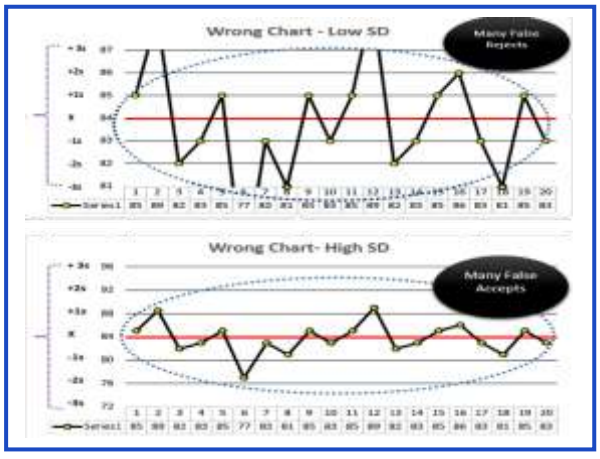
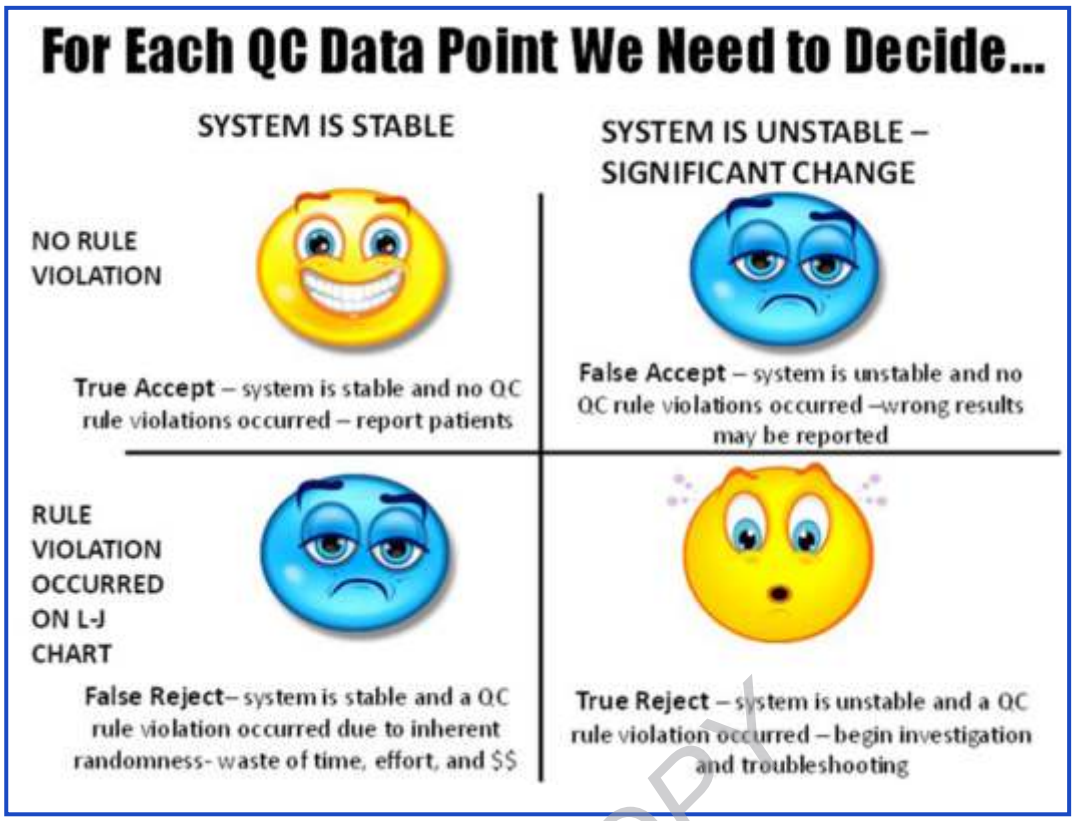


Figure 30: Importance of assigning mean & SD correctly on LJ graphs

Please refer to exercise no.9



OPTIMIZING THE PERFORMANCE OF QC PROCEDURES: 3rd AND 4th GENERATION QC

2.5 Total Error (TE)

2.5 (a) Total Error (TE)

TE is evaluating the combination of errors. Total error combines bias and imprecision to quantify the largest variation from the true or target value. Total analytical error is a useful metric both to assess laboratory assay quality and to set goals. The common evaluation methods are:

Direct Estimation

Indirect Estimation: (Discussed here)

Simulated Estimation

Indirect Estimation is by combining imprecision (SD) and average bias in the equation:

Total analytical error = SE (bias) + RE (1.65 * imprecision). Total Error thus will decrease if the SE component (Bias) of RE component (SD) decreases and vice versa. It provides a simple, cost effective method for evaluating performance.

2.5 (b) Target Value / True Value

Target Value may be obtained from

1) Inter-laboratory comparison programs of the QC manufacturer. Good QC providers give monthly as well as cumulative means. The cumulative means are robust values and will give very good anchoring as the true value
2) Manufacturer assigned mean
3) Long term lab mean provided the QC lot has been running for a considerable duration

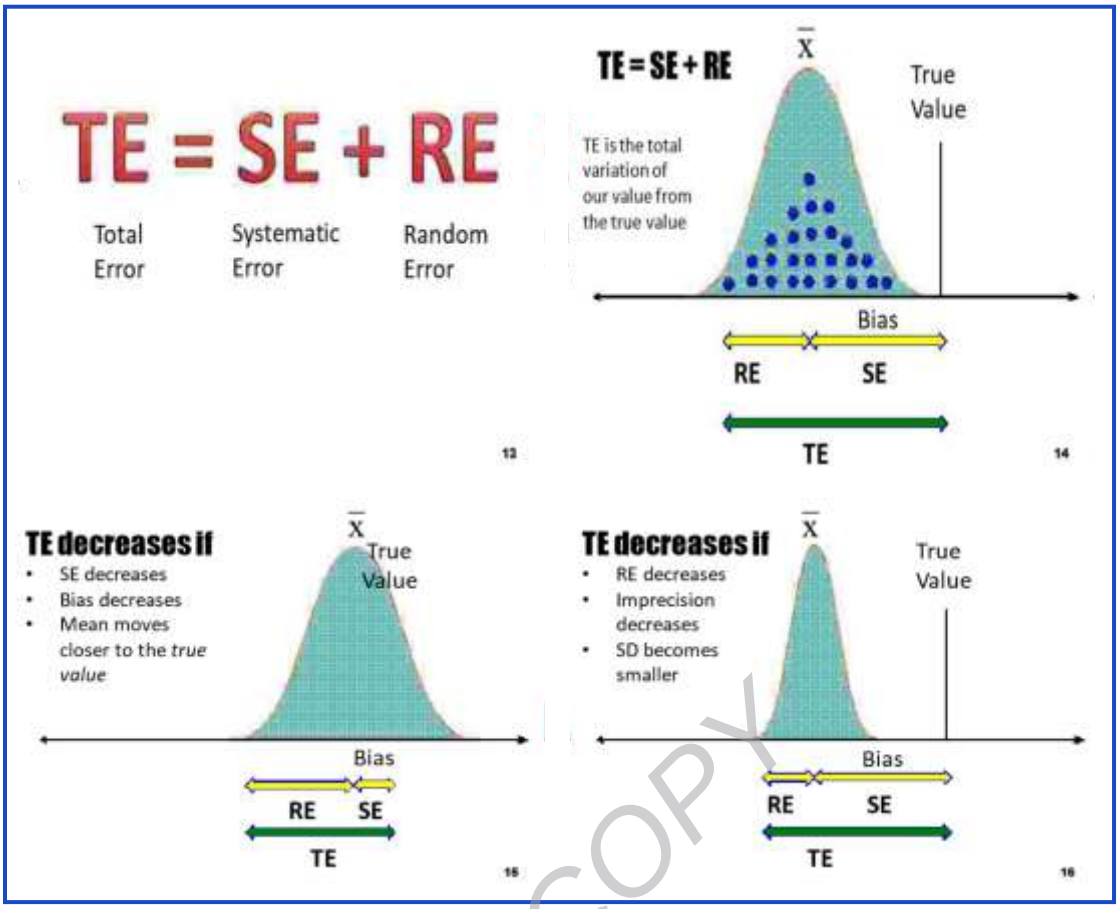


Figure 31: Concept of Total Error (Combination of Systematic and Random Errors)

2.5 (c) Systematic Error (SE) or Bias

Bias is the difference obtained by subtracting the target value from the lab mean value. Bias has direction. If the mean is more than the target it is a positive number and if less, a negative number. But for the sake of calculations, the absolute number has to be used.

Example: If the Target is 100, and the Mean is 95, the Bias is 95- 100= -5. The absolute bias [bias] is 5. SE is the absolute bias. The Systematic Error or SE here is 5.

Please refer to exercise no.10

2.5 (d) Random Error (RE)

We have seen in the above discussion that errors in precision affect method performance and is measured as SD or CV%. Random Error is computed imprecision. Analytical errors need to capture the degree of randomness in a measurement. There are 6 SDs (population of data points) covered under the Gaussian, 3 on each side of the mean. **± 3 SD captures 99.7% of the data points. ± 2 SD captures 95% and ± 1.65 SD captures 90% and ± 1 SD 68% of data points.** It can be understood from the figure 32, 50% of the population of data points (the half of the Gaussian between the target and the Mean, X-bar) are already captured along with the bias. A 1.65 SD will capture 90% from both sides, leaving out 5% on each tail. But since one side is already accounted for, 1.65 is now effectively capturing 95% of the total randomness (figure 32 and figure 33). Most analytical error calculations use 1.65 as the Z factor for capturing random error.

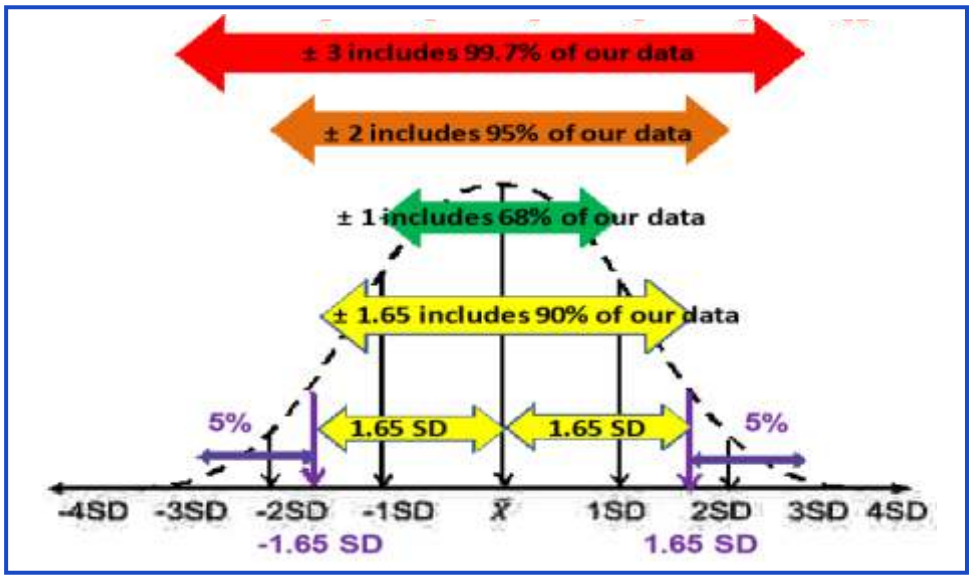


Figure 32: Capturing random errors from a Gaussian. The rationale of using 1.65 as the Z factor.

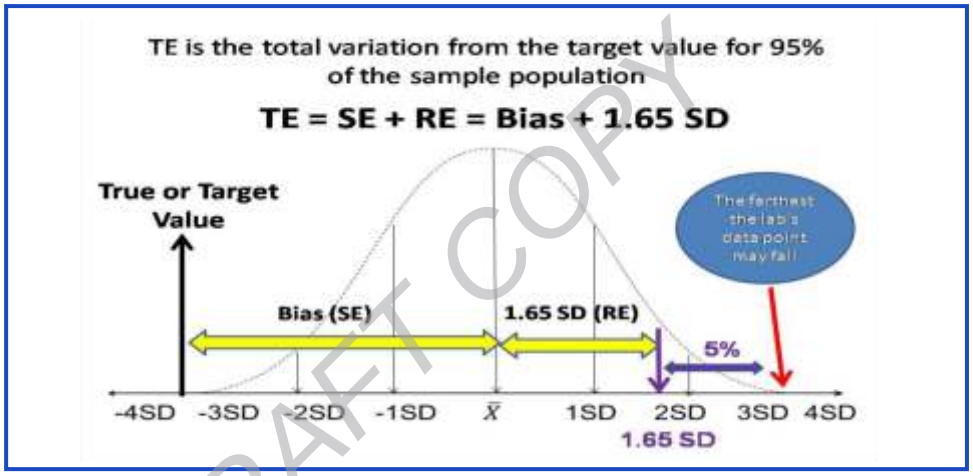


Figure 33: Capturing TE from a Gaussian. 95 % of the error are detected by using 1.65 as a z factor

2.5 (e) The Z Factor

The Z Factor determines the portion of the population of data points to be included in the estimation of the TE. The common multipliers with the SD are:

- 2, Commonly used for quick calculations
- 1.96, to include 97.5% probability
- 1.65, to include 95% probability

For example, using 1.96 as the Z factor, 97.5% of the possible data points will fall within the TE attributed. 2.5% of the possible error points will not be captured. On a more practical note, 1.65 is used as the Z factor to capture 95% of the randomness.

Classification Probability at Z Standard Deviations from Cut-off

z	Probability Below	Probability Above	-z
3.00	0.999	0.001	-3.00
2.99	0.999	0.001	-2.99
2.98	0.999	0.001	-2.98
2.97	0.999	0.001	-2.97
2.96	0.998	0.002	-2.96
1.99	0.976	0.024	-1.99
1.98	0.976	0.024	-1.98
1.97	0.976	0.025	-1.97
1.96	0.975	0.025	-1.96
1.95	0.974	0.026	-1.95
1.94	0.974	0.026	-1.94
1.93	0.973	0.027	-1.93
1.66	0.951	0.049	-1.66
1.65	0.950	0.050	-1.65
1.64	0.949	0.051	-1.64
1.63	0.948	0.052	-1.63
1.62	0.947	0.053	-1.62
1.61	0.946	0.054	-1.61
1.60	0.945	0.055	-1.60

Figure 34: Z factor Probability Chart

Please refer to exercise no.11

2.6 Total Allowable Error

Knowing the Total Error in a system will be of clinical use only if there is benchmarking for the allowable error for that analyte. Hence in the Total Quality Management system (TQM), the concept of Total Allowable Error (TE_A) is very significant. Hence we can say that there are 4 key numbers required to proceed with the performance evaluation quality specifications.

Figure 35: The Four Key Numbers

2.6 (a) Quality Requirements

Total Allowable Error (TE_A) is the amount of error that can be tolerated without invalidating the medical usefulness of the analytic result. The concept of quality requirements is the foundation for quality planning. Quality requirements can help guide interpretation of laboratory test results because they provide perspective about variability of results within an acceptable interval and potential significance of abnormal findings. A commonly used quality requirement is Total Allowable Error (TE_A), which is derived from medically important analyte concentrations or clinical decision thresholds. A hierarchy of quality requirements has been proposed, and the most stringent quality requirements are based on clinical outcomes and clinical decision thresholds. Quality requirements may also be based on data about biologic variation of an analyte (BV Values), analytical performance criteria of Proficiency Testing guidelines (e.g., as mandated by CLIA), Proficiency testing values, and in the absence of any better published guidelines, Tonk's rule or even current SD * 3. These are explained below;

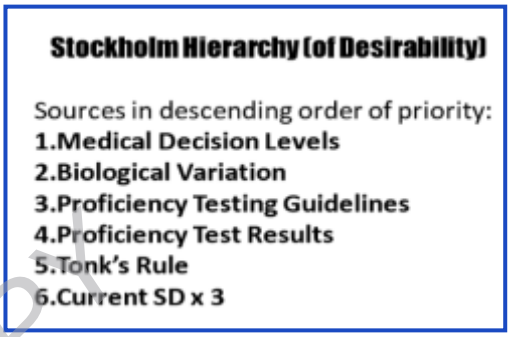


Figure 36: Stockholm Hierarchy for TE_A

2.6 (b) Getting the TE_A values: Applying the Stolkholm Hierarchy

1) Medical Requirements:

Apart from a few analytes like HbA1C with a TE_A specified as as ± 6% by NGSP and Total Cholesterol ± 9%, HDL-C 13%, LDL-C 12%, Tryglyceride 15% specified by NCEP, no other analyte has directly defined TE_A values.

2) Biological Variation (BV) Values

The BV values have 3 categories, Optimum, Desirable and Minimum specifications. The optimum is the most stringent and Minimum. the most lenient. The labs are well advised to find a TE_A that is defendable and attainable and hence start with desirable and up-grade or downgrade as possible.

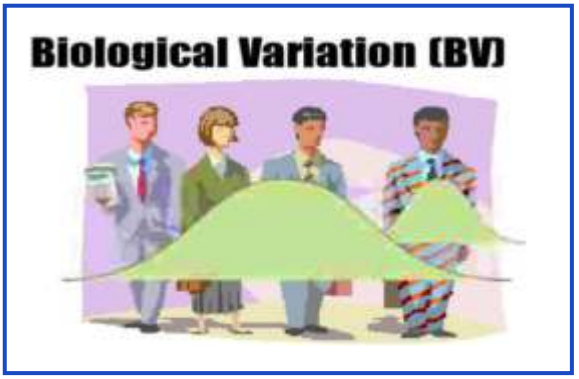
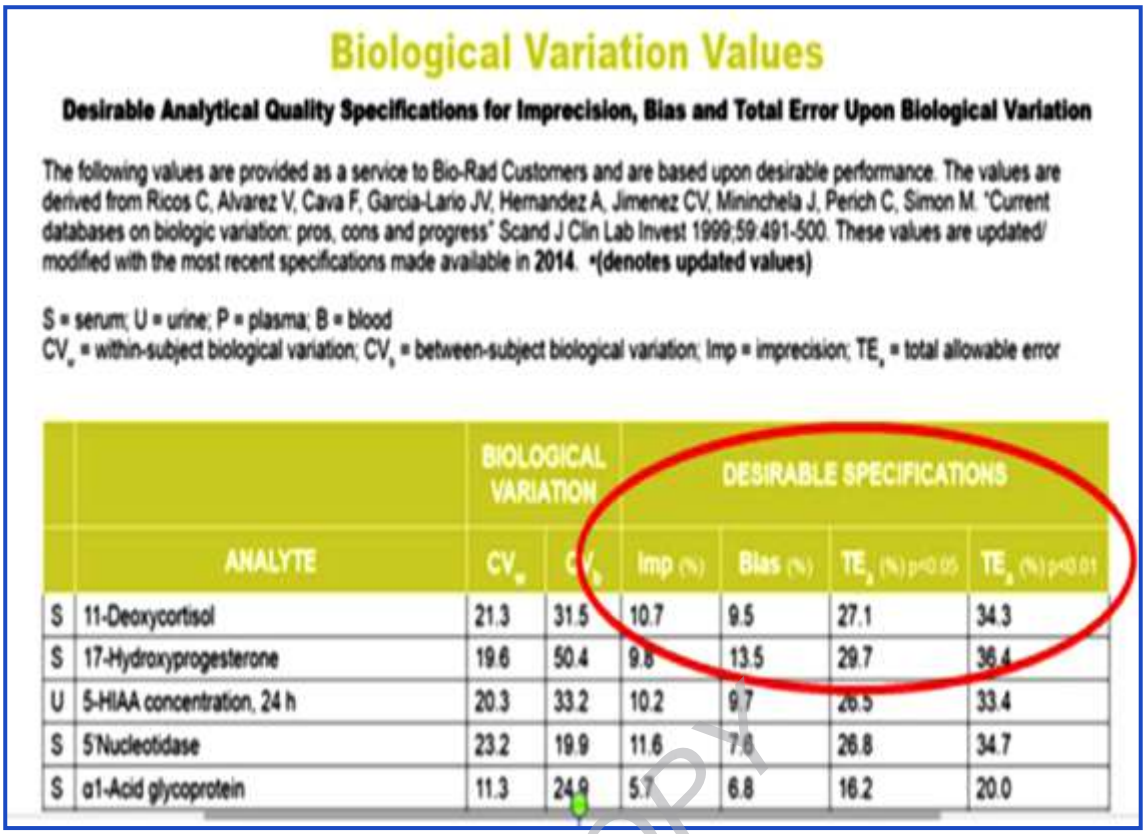


Figure 37: A graphical representation of iIntra and Inter individual BV



Biological Variation Values

Desirable Analytical Quality Specifications for Imprecision, Bias and Total Error Upon Biological Variation

The following values are provided as a service to Bio-Rad Customers and are based upon desirable performance. The values are derived from Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minichela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress" Scand J Clin Lab Invest 1999;59:491-500. These values are updated/ modified with the most recent specifications made available in 2014. ***(denotes updated values)**

S = serum; U = urine; P = plasma; B = blood
CV_w = within-subject biological variation; CV_b = between-subject biological variation; Imp = imprecision; TE_a = total allowable error

		BIOLOGICAL VARIATION		DESIRABLE SPECIFICATIONS			
	ANALYTE	CV_w	CV_b	Imp (%)	Bias (%)	TE_a (%) p=0.05	TE_a (%) p=0.01
S	11-Deoxycortisol	21.3	31.5	10.7	9.5	27.1	34.3
S	17-Hydroxyprogesterone	19.6	50.4	9.8	13.5	29.7	36.4
U	5-HIAA concentration, 24 h	20.3	33.2	10.2	9.7	26.5	33.4
S	5'Nucleotidase	23.2	19.9	11.6	7.8	26.8	34.7
S	α1-Acid glycoprotein	11.3	24.9	5.7	6.8	16.2	20.0

Figure 38: BV Charts (Desirable); An excerpt

Note on abbreviations:

CV_I = within-subject biologic variation

CV_G = between-subject biologic variation

I = desirable specification for imprecision

B = desirable specification for inaccuracy

TE = desirable specification for allowable total error

3) Proficiency Testing guidelines

Performance goals set by organizers of external proficiency assessment programs (e.g. CLIA) may also be used to derive the TE_A values. Most of the participant failures in PT programs were found to be attributable to analytical errors. Although modern analytical instruments are inherently capable of producing results that are accurate and precise enough to meet clinical requirements, the quality-control (QC) practices are not optimized to detect the presence of significant error. In order that QC procedures can ascertain stable equipment performance, CLIA has prescribed the TE_A limits on deviations of from the observations in the PT program and as per the criticality of the analyte. CLIA specifies the goal as percentages or ± absolute values at the target or as ± 3 SD or a combination.

CLIA Proficiency Limits

Analyte or Test	CLIA Criteria for Acceptable Performance
Alcohol, Blood	± 25%
Alanine Aminotransferase (ALT/SGPT)	± 20%
Albumin	± 10%
Alkaline Phosphatase	± 30%
Alpha-1 Antitrypsin	Target value ± 3 SD
Alpha-Fetoprotein (Tumor Marker) AFP	Target value ± 3 SD
Amylase	± 30%
Antinuclear Antibody	Target value ± 2 dilutions or positive/ negative
Antistreptolysin O	Target value ± 2 dilutions or positive/ negative
Anti-Human Immunodeficiency Virus	Reactive or nonreactive
Aspartate Aminotransferase (AST/SGOT)	± 20%
Bilirubin, Total	Target value ± 20% or ± 0.4 mg/dL (greater)
Calcium, Total	Target value ± 1.0 mg/dL
Creatine Kinase	± 30%
Creatine Kinase CK-MB	Target value ± 3 SD or presence/ absence
Creatinine	Target value ± 15% or ± 0.3 mg/dL (greater)
Digoxin	Target value ± 20% or ± 0.2 ng/mL (greater)
Erythrocyte Count RBC	± 6%
Ethosuximide	± 20%
Fibrinogen	± 20%
Free Thyroxine Free T4	Target value ± 3 SD
Gentamicin	± 25%
Glucose	Target value ± 10% or ± 6 mg/dL (greater)
Hematocrit (Excluding Spun Hematocrits) HCT	± 6%
Hemoglobin Hgb, Total	± 7%
Hepatitis (HbsAg, anti-HBc, HbeAg)	Reactive (positive) or nonreactive (negative)
Human Chorionic Gonadotropin Beta	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Intact	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Qualitative	Target value ± 3 SD or positive/ negative

Figure 41: CLIA proficiency limits: Excerpts

Proficiency Testing Guidelines

Performance goals set by the organizers of external proficiency assessment programs (e.g. CLIA in the USA) or in-country regulatory bodies.

CLIA's analytical quality requirements are presented in four different ways, depending upon the analyte:

1. **Percentage**
   Chloride Target Value ± 5%
2. **Absolute concentration limit**
   Potassium Target Value ± 0.5 mmol/L
3. **Distribution of a survey group**
   TSH Target Value ± 3SD
4. **Combination of requirements #1 (for lower concentrations) and #2**
   Glucose Target Value ± 0.3 mmol/L or ± 10%, whicheveris greater

Figure 39: CLIA limits defined in different ways percentage, +/- Absolute values, +/- SDs and combined

Sample (cell/uL)	Mean	SD	%CV
C-1	786.4	32.2	4.1%
C-2	56.0	2.8	5.0%
C-3	491.3	27.5	5.6%
C-4	103.4	6.0	5.8%
C-5	292.8	14.1	4.8%

You decide to use a past survey report to derive a TE_A% for CD4 cell count since there is no other published source available.

What TE_A % will you use?

For a target of 100 cell/uL , what are your lower and upper TE_A limits?

Figure 40: Estimating the TE_A using labs owns proficiency testing limits

4) Using Proficiency Testing Results (past survey report)

In the absence of any guideline, the lab may use the survey reports from earlier PT reports. As median values are used in PT reports, it will be less affected by outliers and hence a good indicator of TE_A. An example is given above (Figure 40). The CD 4 count reports complied shows a certain variation at each level, in SDs and CVs. A 3* SD or CV may be applied as the TE_A. In the figure, if you take the average CV% it will be 5%. Three times this is 15%. This may be applied as the % TE_A. A count of 100 cells, an acceptable would be ± 15. Alternatively, the lab can use 3 times the respective CV% against each level.

5) Tonk's Rule: TE_A from Biological Reference Intervals

TE_A = 25% * BRI as per Tonk's rules. Subtract the lower end of biological reference range from the upper end and divide by 4 for the absolute number or derive the percent with the target value as the denominator. TE_A from reference intervals are also referred to in CLIA '88 rules which suggests 50% * BRI. This gives rise to considerable problems(See fig). Besides, the reference intervals are lab defined, often revised. So it is ideal to avoid this.

6) Current Lab (Observed) % CV *3

As a last resort, the current CV can be taken as a guideline. 3 times the CV will not only accommodate the random error, but account for the SE also.

TEa from Ref Intervals – Example

Calcium TEa (mg/dL)

Reference Interval	Range	Tonks Rule 25% of Range	CLIA Rule 50% of Range
8.5 – 10.5 (Traditional)	2.0	0.5 mg/dL (0.5/9.5 = 5.3%)	1.0 (10.5%)
8.9 – 10.1 (Recent)	1.2	0.3 (3.2%)	0.6 (6.3%)

Figure 42: Estimating TE_A using reference values (Tonk's Rule)

2.6 (c) Uses of TE_A

TE_A can be used to aid

1. Instrument selection if manu-facturer's claims such as CV/ SD for Medical Decision Points for instrument performance are available.
2. TE_A can also use for method evaluation to determine whether that instrument's analytical performance is adequate.
3. If analytical performance is deemed adequate, TE_A can further be used during ongoing performance evaluation.
4. TE_A can be used to guide comparison of test results across laboratories and clinics using the same or different analytical methods.
5. TE_A can be used to help interpret results from external quality assurance (proficiency testing) programs or to help interpret results of comparability testing, where a reference laboratory is used to "check" in-clinic or other laboratory results.

Tools for 1-3 are available in the Labs for Life website. 4 &5 can also be analyzed using the method valuation tool.

It is important to realize that TE_A may differ with analyte concentration - TE_A may differ at low, or high analyte concentrations.

Additional information about TE_A can be found in [CLSI- C54-A, 2008].

Please refer to exercise no.12

2.7 How Far Can the Mean Shift?

Once the TE and TEA are known, it is possible to assess the error margin. There are different methods in which this can be done.

2.7 (a) Margin of Error

In the figures below, the observed mean is less than the True or Target Mean, rendering the 1.65 SD or the outer edge of the Gaussian, towards the lower end of TEA. But if the mean shifts further to the lower end as in figure below, 1.65 SD will touch and then cross the lower limit of the TEA. This is the margin of error. In calculation, the margin for error can be considered as TEA minus TE. If TE approaches TEA the margin for error decreases. If TE exceeds TEA, the analytical system may be considered invalid .

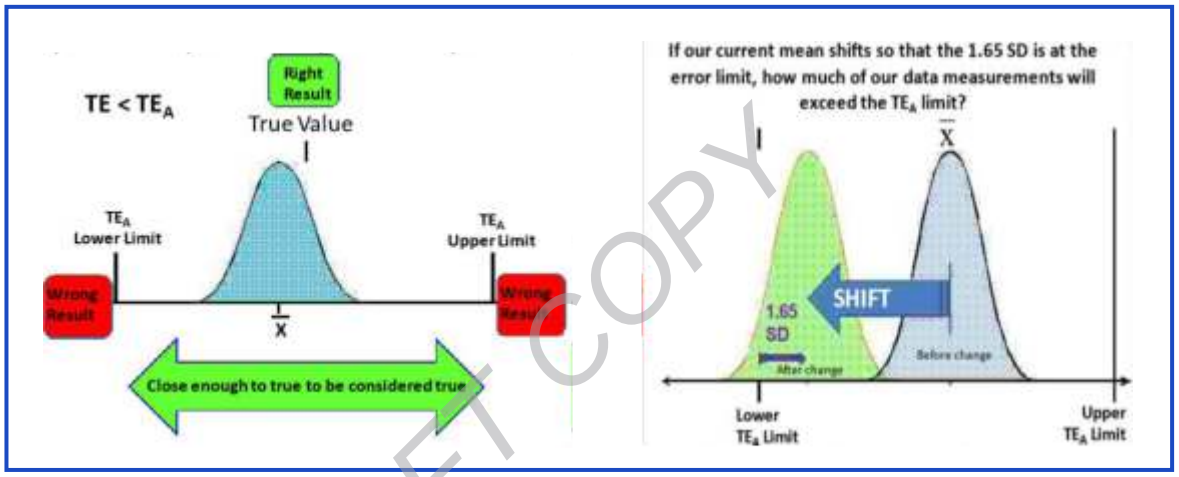


Figure 43: (a) TE< TE_A, (b) TE>TE_A

2.7 (b) Critical Systematic Error (SEc) or Δ SEcrit

SEc is the size of the systematic error that needs to be detected to maintain a defined quality requirement. Critical Systematic Error or SEc is the number of SDs the mean can shift before exceeding the TE_A. Thus SEc quantifies the Margin of Error in terms of a measureable parameter, the SD. It measures the multiples of SD that fit within TE_A limits.

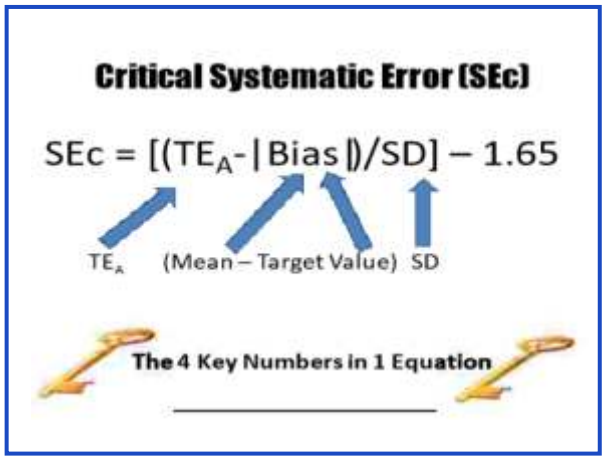


Figure 44: Calculating SEc using the four key numbers & Z factor

The calculation of SEC also makes use of the 4 key numbers

{(TEA- Absolute Bias) / SD} – 1.65

{(%TEA -% Bias)/ % CV}-1.65.

Where 1.65 is the Z factor and represents the tail of the histogram that exceeds TE limit. By using this Z factor, we are taking on a risk of 5% of wrong reports as acceptable.

2.7 (c) Sigma-metric = [(TE_A – Bias)/SD] = [(%TE_A -% Bias)/ % CV] = SEc + 1.65

We may consider the 1st generation QC as Levey-Jennings for manual methods and 2nd generation QC for automated analyzers. Optimizing the performance of QC procedures on the basis of quality required for the intended use of the test in terms of Total Error observed for a particular method and definition of Total Allowable Error may be considered the 3rd and 4th generation Qcs.

The 5th general QC emphasizes the need to define "tolerance limits" to describe intended use, set a goal of 6-sigma for "world class quality," and provides a uniform way of describing quality in terms of defects, defect rates, defects per million (DPM), and the sigma-scale itself. A world class or Six Sigma performance makes less than 3.4 defects per million operations. ISO mandates that labs capture their quality indicators as % Yield, % Defects, Defects per Million Occasions (DPMO) or Sigma. QC monitoring is by far, the most vital Quality Indicator of a lab. A "sigma-metric QC selection tool" readily evolved from an earlier "critical-error" tool and was eventually included in the CLSI C24A3 guidance for "Statistical Quality Control for Quantitative Measurements". Thus, standard QC planning tools became available in the forms of manual tools, as well as computer programs.

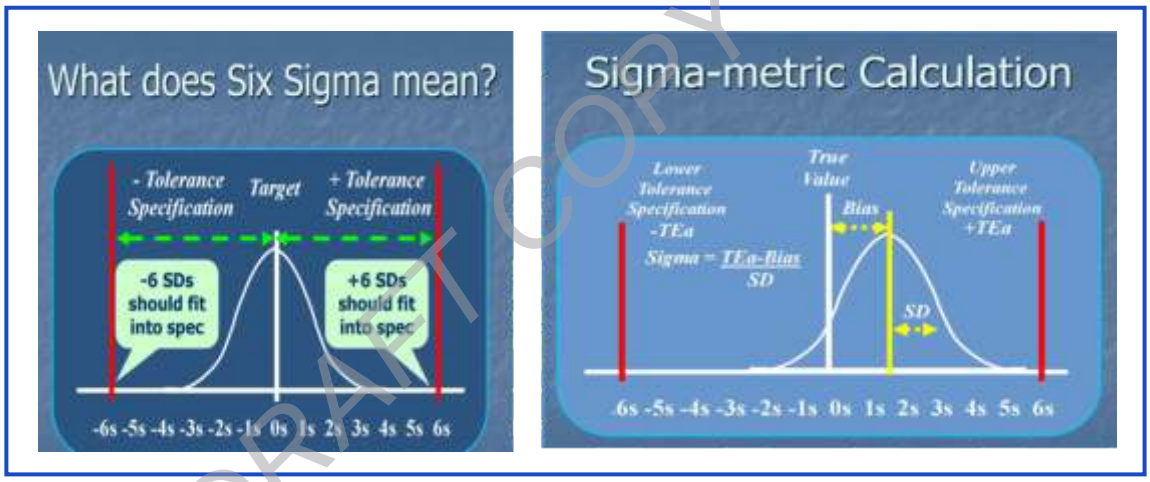


Figure 45: Concept of Six Sigma in laboratories

A six sigma test can fit in 6 SDs on either side of the mean. This means there is no bias and the degree of dispersion or imprecision is stable. In figure 45, see the first picture. This is Six Sigma performance where the chances of defects is < 3.4/ million.

In the second picture, the positive bias formation has compromised the margin the mean can shift, and the Sigma number. Similarly a widening Gaussian due to imprecision can also breach the limits leading to lower Sigma.

Deriving the sigma metric for an analysis again combines the 4 Key Quality Numbers; Mean, SD, Target and TE_A into one statistic. It benchmarks the performance of the measurement procedure in relationship to the quality required (i.e. Six Sigma). Knowing the Sigma performance of an analyte can be used to select appropriate control rules for a method. . This is described in later sections.

Sigma = (TE_A - Absolute Bias) / SD

or

Sigma = (%TE_A -% Bias)/ % CV

Example:

Mean obs = 15 mmol/L

SD obs = 3 mmol/L

Target Value = 18 mmol/L

TE_A = 15 mmol/L

Sigma = (15- 3)/ 3 = 4

Thus essentially the sigma metrics is an extension of SEc but can also be called a sigma-metric, which is more easily understood in light of current interests in Six Sigma Quality Management. Depending on the sigma performance on an anlayte, the monitoring rules for that analyte can be modified. See Figure 46

Please refer to exercise no.13

Sigma Performance Table

If	Then
The Sigma metric less than 2.0	• The method has **unacceptable performance** and does not meet your requirement for quality, even when the method is working properly. • It is not acceptable for routine operation.
The Sigma metric is between 2.0-3.0	• The method has **marginal performance** and provides the necessary quality when everything is working correctly. • This method will require: o 4-8 controls per run o well-trained operators o reduced rotation of personnel o more aggressive preventive maintenance o careful monitoring of patient test results o continual efforts to improve method performance
If the Sigma metric is between 3.0-4.0	• The method has **fair performance** and meets your requirement for quality and can be managed in routine operation. • This method will require a multirule procedure with 4-6 control measurements per run.
If the Sigma metric is between 4.0-6.0	• The method has **good performance** and is clearly acceptable and can be well-managed in routine operation with 2-4 control measurements per run, using standard Westgard QC rules.
If the Sigma metric is >6.0	• The method has **Six Sigma performance** and can be managed using a single control rule with wide limits (i.e. 1:3s, 1:3.5s).

Figure 46: Sigma Performance matrix

HOW TO USE ALL THESE INFORMATION IN YOUR LAB

2.8 QC Planning

The outlines of statistical QC may be evident from the above discussions. With the knowledge of the basics, it is time to understand how to make a QC protocol for each analyte depending on the method performance.

An effective QC design

1. Ensures quality performance by quickly detecting medically significant errors (Percent Error Detection or P ed > 90%);
2. Generates few rule violations when there are no significant errors occurring (Percent false rejection; P fr < 5%);

3. Fewest number of control measurements per analytical run possible to save on costs associated with;
   - QC materials
   - Reagents
   - Consumables
4. Meets regulatory or accrediting body's requirements for number of measurements per run.

2.8 (a) Percent False Rejects and Percent Error Detection

A simple Westgard rule system 1:2s (mean ± 2 SD) was used initially to monitor method performance. However this rule has Percent False Rejection (P fr) of 4.5% in one control measurement and 9% at 2 control measurements. This high false rejection rate would render this rule a major waste of laboratory resources due to repeat analysis of controls and samples resulting in an increase in the cost of the analytical process and a waste of time and effort.

This type of waste can be avoided by designing a quality control procedure that is based on the quality goal required clinically and the performance characteristics of each test/analyzer. The laboratory's efforts would be focused on the analytes that require the maximum control. The ideal IQC design should be derived for each individual test in a multi-test system, selecting where possible the combination of the highest Percent Error Detection (Ped) and the lowest Percent False Rejection (P fr).

2.8 (b) Using Multi-Rules: Seeing the Complete Picture.

A single-rule QC procedure uses a single criterion or single set of control limits, such as either the mean plus or minus 2 standard deviations (2s) or the mean plus or minus 3s. Multi-rules QC on the other hand, uses a combination of decision criteria, or control rules, to decide whether an analytical run is in-control or out-of-control.

The N and R

N represents the total number of control measurements that are available at the time a decision on control status is to be made. If 2 levels of (control levels)measurements are available within one run, N=2. If three are available then, N=3.

R represents the number of runs

Example: If 2 levels of control measurements are available and two runs are available , N=2 & R=2 and the total available data points are 4 per day.

1:2s rule may be used as a warning to trigger application of the other rules, thus anytime a single measurement exceeds a 2SD control limit, respond by inspecting the control data using the other rules.

Within Run Errors: The Power of Daily Monitoring

- Stop and take corrective action if a single point exceeds a 3s limit.
- Stop and take corrective action if two levels of control exceed the same 2s limit.

- Stop and take corrective action if one point in the group exceeds a plus 2s limit and another exceeds a minus 2s limit: R_4s This is a range rule that is meant only to be applied within-run

Because N must be at least 2 to satisfy CLIA QC requirements, all these rules can be applied within a run.

Across Run Errors: The Power of Periodic Review

Several rules like 4:1s, and 10x must be used across runs, within or across materials in order to get the number of control measurements needed to apply the rules and to pick up systematic errors. 2:2s can be used within and across runs. . In the case of 7 T, whenever one level is trending, say, upward for 5- 6 times, and other level doing the same thing, it should be investigated.

To reiterate, the advantages of multi-rules QC procedures are that false rejections can be kept low while maintaining high error detection. This is done by selecting individual rules that have very low levels of false rejection, then building up the error detection by using these rules together

The power of daily monitoring PLUS The power of periodic review = $1_3s/2_2s/R_4s/4_1s/10x$

For certain types of tests, notably hematology, immunoassay and blood gas, controls tend to be run in three's, i.e., one low control, one middle control, and one high control. For situations like this, it isn't practical to use the "Classic Westgard Rules"; those rules were built for controls in multiples of 2. So when you're running 2, 4, 8 controls, use the "classic" rules. When you're running 3 or 6 controls, use a set that works for multiples of threes: In this case:

The power of daily monitoring PLUS The power of periodic review =1_3s/2 of 3_2s/R4s/ 3_1s/12x

2.8 (c) Length of Analytical Run

The length of an analytical run must be defined appropriately for the specific analytical system and specific measurement procedure. In laboratory operations, control samples should be analyzed during each analytical run to monitor method performance. The length of the analytical run can be defined as an interval over which the risk (severity and likelihood) of unexpected events that could impact precision and accuracy has been mitigated to a tolerable level by virtue of the operational characteristics of the testing system. The user should define the run length for the specific application in their own laboratory because the operating conditions, workload, and application of the measurement procedure in their laboratory may differ from nominal conditions evaluated by the manufacturer.

The user should define the period of time or series of measurements within which validation of the measurement procedure is important, based on the expected stability of the measurement procedure, the number of patient samples typically being analyzed, cost of reanalysis in the event of a QC failure, workflow patterns, operator characteristics, and the clinical impact of an undetected error condition existing for a period of time before the next QC measurement(s). Stability of an analyte in patient samples is a consideration

because if an out-of-control condition is identified, then it is important that the QC frequency will allow for the retesting of all potentially affected patient samples.

2.8 (d) Frequency of Control Measurements

Quality control samples must be analyzed at least once during each user-defined analytical run length. Manufacturers of analytical systems or reagents may recommend the number of quality control specimens and their location within the run. However, manufacturers' recommendations should be used as guidelines and the frequency of QC measurement should be established by the laboratory considering the factors outlined later. The frequency and location of control samples should reflect actual test system performance and application at the site of testing.

2.8 (e) Location of Control Samples

The user should determine the location of control samples within a run, keeping in mind the principle that quality control results should be evaluated before reporting patient results from the run. The location of control samples should consider the type of analytical process, the kinds of errors that might occur, and the protocol for reporting patient results. For example, if an analytical run corresponds to a discrete batch of samples, the controls might be located at the beginning and the end of the run to detect shifts, might be spaced evenly throughout the batch to monitor drift, or distributed randomly among the patient samples to detect errors. In any case, the QC results would be evaluated before patient results are reported. For a high-volume analyzer that continuously produces test results, an appropriate analytical run might be defined as a certain interval of time, then QC samples would be analyzed and evaluated at the beginning of a run and then again as each run (i.e. ., the next time interval or defined number of samples) occurs. If a quality control fault is detected, results reported since the previous quality control event should be reviewed.

CAUTION: Routine placement immediately after calibration materials may give falsely low estimates of analytical imprecision and will not provide any estimate of shift or drift during the run.

2.8 (f) Developing a QC Plan

As explained earlier to come to the sigma-metrics, they 4 key numbers should be available, the precision (SD/ CV) and accuracy (Observed Mean and Target Value) and TE_A. Calculate the SEc and Sigma. It is important to have internal QCs with target values near Clinical Decision Points.

Define the quality required for each test, then assess the probabilities for false rejection (P fr) and error detection (P ed) of the different candidate QC procedures on the Rule Selection power graph. Aim for 90% error detection (P ed of 0.90 or greater) and 5% or less false rejections (P fr of 0.05 or less).

Aim for 90% detection of medically important errors,

5% or less false rejections

To recap, use the following steps are suggested to develop an optimum QC plan

1. Define the quality that is needed for each test.
    a. Know the performance of your method (CV, bias).
    b. Get target value and Total Allowable Error from the best possible source.
    c. Calculate the SEc and Sigma-metric of your testing process.
2. Decide on the rules to be applied to each analyte from the Rule Selection Power Graph; Single Rule/ Multi rules.
3. Decide the number of controls measurements(N) and (R), number of runs of each QC
    a. Use single-rule QC procedures and minimum number of control measurements (N) & (R) for methods with high performance
    b. Use single-rule QC procedures and moderate number of control measurements (N) & (R) for methods with moderate to high performance
    c. Use multirule QC procedures for methods with moderate to low performance
4. Define explicitly the application and interpretation of rules within and across materials and runs
5. Interpret multirule to help indicate the occurrence of random error or systematic error.

2.8 (g) Tools to Use to Determine the Appropriate Control Rule(s)

If medically important errors can be detected 90% of the time (i.e., probability of error detection of 0.90 or greater), then a single rule QC procedure is adequate. If 90% error detection cannot be provided by a single rule QC procedure, then a multi-rules QC procedure should be considered. In general, single rule QC procedures are adequate for highly automated and very precise chemistry and hematology analyzers. However, the 2s control limits or the 1:2s control rule should be avoided to minimize waste and reduce costs. Earlier generation automated systems and manual methods will often benefit from the improved error detection of multi-rules QC procedures.

There are many tools available to understand the rule(s) that should be used to alert you to a significant error. The tools include power function graphs such as Sigma-Metric Rule Selection Tool, critical-error graphs, QC Selection Grids, charts of operating specifications (OP Specs chart), and the QC Validator, Westgard advisor by Bio-Rad, Optmizer and EZ Rules .

In this manual, Sigma Metric Rule selection tool will be explained as it tis the tool described in CLSI guidelines.

2.8 (h) Using the Sigma Metric Rule/Rules Selection Tool

This tool is a power function graph that shows the probability for rejection vs. the size of the error for different QC rules and numbers of control measurements. The key to this tool is the critical systematic-error (SEc) that needs to be detected by the QC procedure. The rule selection depends on the quality required for the test and the precision and accuracy observed for the measurement procedure. The critical systematic error is shown on the x-

axis at the bottom of the graph, the sigma-scale is shown at the top of the graph, and the probability for error detection and probability of false rejection is shown on the y-axis. The vertical lines represent measurement procedures having 3-sigma, 4-sigma, and 5-sigma performance. The key at the right identifies the QC procedures. The curves in the graph, left to right (1-8), match the list in the key, left to right (1-8). Pfr, probability for false rejection; N, total number of control measurements; R, number of runs to which the QC procedure is applied. Full formats of Tool selection graphs are given as **annexure number 4 A & B**

The steps to be followed are:

1) Locate calculated sigma-value on Sigma-metrics graph.
2) Draw vertical line to intersect power curves.
3) Locate the point at which any of the 8 graphs cross the 0.9.
4) Select QC procedure corresponding to the number of that graph.
5) This set of rules provides Ped of 0.90 or 90% error detection.

In the example below, a SEc of 2.5 (Sigma 4.15) is being evaluated for appropriate QC rules. The graph that intersects at 0.9 or 90% closest is graph number 3. (Please note graphs 3 and 4 crossing over near 0.6 of the Y axis). The set of rules appropriate for Graph 3 is: 13s/22s/R4s/41s, N4 and R1. This means multirules as stated above, for 4 controls available at each run, for 2 runs, and a false rejection of 0.03 or 3%.

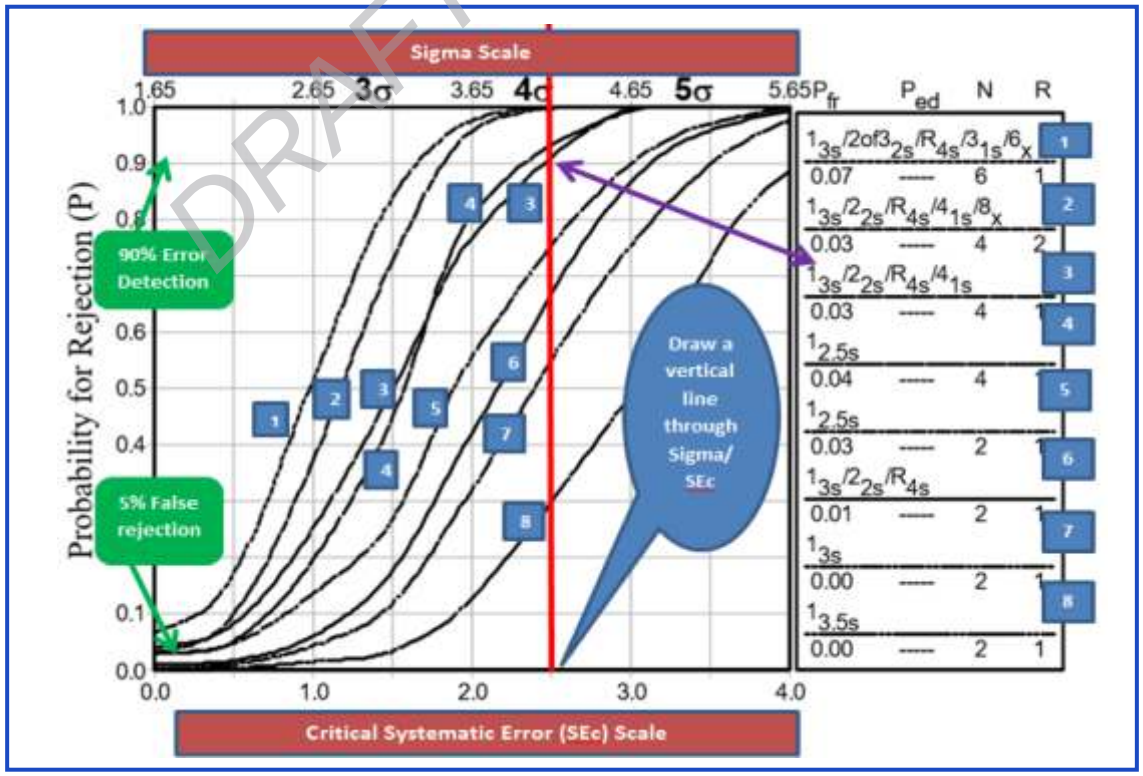


Figure 47: Technique for using Sigma rule selection tool for QC rules in the lab

Recap: The different lines represent the "power" of different QC rules and different numbers of control measurements per analytical run. These QC procedures are identified in the key at the right side of the graph. The power curves left to right correspond to the control procedures listed in the key top to bottom. In situations where the power curves for two different QC procedures are so close they are hard to tell apart; example, power function graphs 3 and 4. In these situations, the user should select whichever QC procedure is more practical to implement (e.g., a single rule may be preferred over multiple rules); a minimum N of 2 may be required by regulations, even though an N of 1 QC procedure may provide the same error detection.

Please refer to exercise no.14

2.8 (i) Using the Westgard Sigma Rule/Rules Selection Tool

https://www.westgard.com/westgard-sigma-rules.htm may be checked to apply the Westgard sigma rules. A brief overview is given below. The yellow lines that come up from the Sigma Scale show which rules should be applied based on the sigma quality determined in your laboratory. The notation N=2 R=1 indicates that 2 control measurements are needed in a single run.

6-sigma quality requires only a single control rule, 13s, with 2 control measurements in each run one on each level of control.

5-sigma quality requires 3 rules, 1:3s/2:2s/R:4s, with 2 control measurements in each run (N=2, R=1).

4 -sigma quality requires addition of a 4th rule and implementation of a 1:3s/2:2s/R:4s/4:1s multi-rule, preferably with 4 control measurements in each run (N=4, R=1), or alternatively, 2 control measurements in each of 2 runs (N=2, R=2), using the 41s rule to inspect the control rules across both runs. This 2nd option suggests dividing a day's work into 2 runs and monitoring each with 2 controls.

<4-sigma quality requires a multirule procedure that includes the 8x rule, which can be implemented with 4 control measurements in each of 2 runs (N=4, R=2) or alternatively with 2 control measurements in each of 4 runs (N=2, N=4). In the first option 4 control measurements are plotted. To determine if the run is acceptable, the frontline worker must examine the current run and the previous run (R=2).The second option is 2 control measurements. The frontline worker examines the current run and the previous 3 runs. (R=4) to determine if the current run is acceptable.

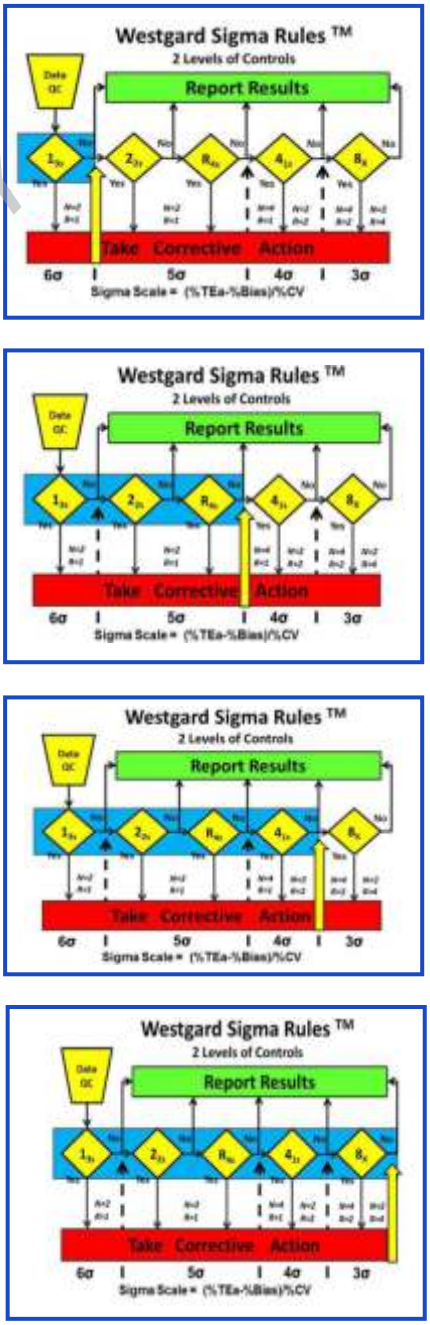


Figure 48: Westgard rule selection tools (EZ rules)

2.8 (j) Using the OPSpecs Tool

Relationship between the quality requirement for the test, the precision and accuracy observed for a method, and the rejection characteristics for different control rules and numbers of control measurements are used to develop OpSpecs Chart. "Normalized" OPSpecs charts minimize the number of charts when the observed imprecision and bias are expressed as a percentage of the quality requirement. Online OpSpecs tools are available for rule selection.

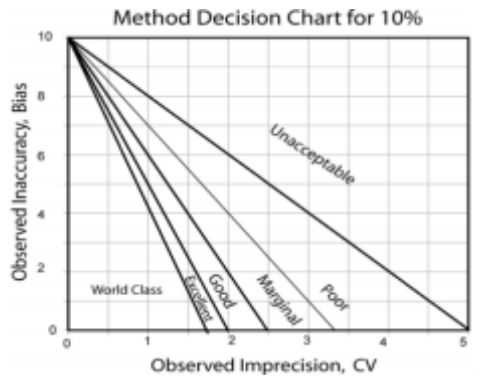
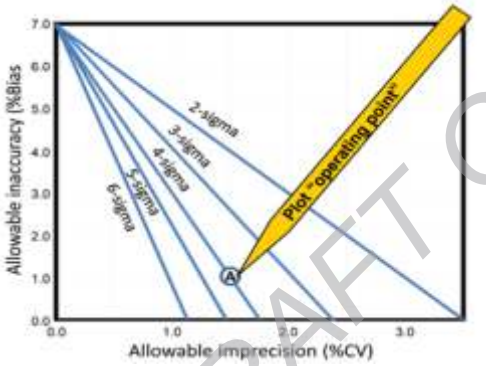
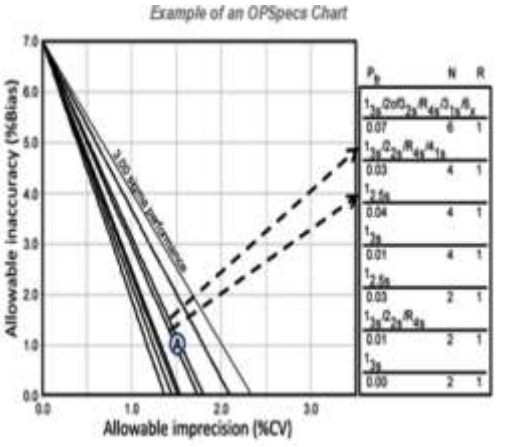
	An OpSpecs chart showing acceptability of the method based on sigma metrics. In practice, the normalized OPSpecs charts are scaled from 0 to 100% on the y-axis and 0 to 50% on the x-axis and the x-coordinate and y-coordinate are expressed as a percentage of the quality requirement
	This example of a methods decision chart shows allowable total error. Allowable inaccuracy (% bias) is plotted on the y-axis versus allowable imprecision (% CV) on the x-axis. Diagonal lines represent, from left to right, 6-sigma, 5-sigma, 4-sigma, 3-sigma, and 2-sigma quality. Operating point (A) shows a method having a bias of 1.0% and a CV of 1.5% that demonstrates 4-sigma quality.
	User plots a normalized operating point that displays method performance as a percentage of the quality requirement of the test. An acceptable QC procedure is one whose operating limits for inaccuracy and imprecision include the user's operating point, i.e., that point falls below the line, whose rules and total number of control measurements are shown in the key at the right.The lines below the 3.0 sigma line represent different SQC procedures, as identified in the key at the right.

Figure 49: OPSpecs scale for Lab QC rule selection

2.8 (k) When Sigma and SEc are low

When an analyte shows <4 sigma, the lab must take special care for risk analysis.

a) SQC Measures

Multi rules

Look-back to previous runs

Increase N: Number of Qcs

Increase R: Number of QC runs

b) Non SQC Methods

Staff with special training to be deployed for low sigma tests

Increase the number of supervision

2.9 Uncertainty of Measurement (Mu)

5.5.1.4 Measurement uncertainty of measured quantity values

The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty.

NOTE 1 The relevant uncertainty components are those associated with the actual measurement process, commencing with the presentation of the sample to the measurement procedure and ending with the output of the measured value.

NOTE 2 Measurement uncertainties may be calculated using quantity values obtained by the measurement of quality control materials under intermediate precision conditions that include as many routine changes as reasonably possible in the standard operation of a measurement procedure, e.g. changes of reagent and calibrator batches, different operators, scheduled instrument maintenance.

NOTE 3 Examples of the practical utility of measurement uncertainty estimates might include confirmation that patients' values meet quality goals set by the laboratory and meaningful comparison of a patient value with a previous value of the same type or with a clinical decision value.

2.9 (a) Why and What is MU?

Clinicians compare most measurement results with reference values and with previous results from the same patient. Results should therefore be reliable and accurate. But the inherent errors could be misleading, rendering ongoing monitoring by clinicians difficult. The MU approach focuses on identifying the dispersion of results that might have been obtained for an analyte if a sample had been measured repeatedly instead of once. CLSI defines MU as associated with the result of a measurement, that characterizing the dispersion of the values that could reasonably be attributed to the analyte. To do this, the MU approach uses available data about repeated measurements from a given measuring system to define an interval of values within which the true value of the measured analyte is believed to lie, with a stated level of confidence. The parameter may be, for example, a standard deviation. **The term measurement uncertainty tends to give the wrong impression, as it is actually a quantitative indication of the level of confidence, or belief, the laboratory has about the quality of a result.**

ISO 15189 mandates the determination of measurement uncertainty of all measurement procedures. All types of measurement that have a magnitude expressed as a number and a reference need to define the MU as per ISO.

2.9 (b) Sources that Contribute to Uncertainty

- Biological within-subject Biological Variation stress, drugs,
- Pre-analytical: including sampling, sample preparation and sample portion and sample transport and selection among
- Analytical: calibrators and reference materials, input quantities, equipment used, changes of operator, water quality and environmental conditions leading to random and systematic errors (RE + SE)
- Post-analytical: such as errors of transcription

As per ISO the Measurement Uncertainty need to factor in only the uncertainty components associated with analytical errors.

2.9 (c) Deriving Uncertainty of Measurement

Ideally the MU should capture all the elements of uncertainty. But as said earlier, in practice, MU is concerned with only analytical uncertainty. In the analytical errors, unlike TE, MU is not concerned with measurement error, but is concerned only with reporting to clinicians. All components of analytical uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion should ideally be included. However, the calculations of MU assumes that the bias cannot be estimated correctly and hence is not considered in the estimate of MU. The MU approach assumes that known bias is eliminated or minimized e.g. by re-calibration. Further, just as a bias value cannot be exactly known, bias cannot be completely eliminated. The MU approach recognizes that the value used for bias correction has an associated uncertainty, being the combination of the uncertainty of the reference value itself.

Thus some of the components like imprecision of the measuring system may be evaluated from the statistical distribution of the results of series of measurements and can be characterized by standard deviations. The other components, like imprecision of the bias value used if bias was eliminated or minimized may also be characterized by standard deviations as expanded uncertainty. Since it is rarely possible in practice due to limited time and resources, the extended uncertainty of a measurement result is usually evaluated with a mathematical model using the law of propagation of uncertainty.

2.9 (d) Combined Standard Uncertainty (μc)

As the best material that lends itself to repeated analysis is Internal Quality Control, ISO suggests the IQC values form the basis of MU determination. The mean value and SD is calculated for each level of QC used for a given measurement procedure over a sufficient time to encompass as many routine procedure changes as possible; at least 30 values is be adequate for an initial MU estimate. The parameter of MU is 1 SD (standard measurement uncertainty, symbol μ). Because the SD of the QC reflects the combined effect of all the individual uncertainties arising within the measuring system, the SD can be considered as the combined standard uncertainty (μc) for patients results around the mean value of the particular QC.

2.9 (e) Expanded Uncertainty (U)

Since ±1 SD covers only ~68 % of the dispersion of obtained QC values, the uncertainty is widened by applying a coverage factor (k) to provide an expanded measurement uncertainty (symbol U). Usually k = 2 is chosen, to **provide a more useful 95.5 %** coverage of the dispersion of results. Assuming such a dispersion also applies to patients results, then a result could be in the form x ± y (95 % confidence), where x is the result obtained from the test and y = 2 SD (i.e. 2 x μc = U).

If several levels of QC are used the MU should be calculated for each, and a judgment made as to whether they are sufficiently different to warrant their use with patient results that fall in the range considered to be covered by each QC level.

The expanded UM may also be expressed as a percentage at that level, which is the CV%* 2.

2.9 (f) Coverage factors and confidence limits

Applying the following coverage factors will offer the corresponding confidence limits.

- k=1.00 (68.27%)
- k=1.00 (64.90%)
- k=1.00 (96 .95%)
- k=2.00 (95.45%)
- k=2.00 (58 99%)
- k=3.00 (99.73%)

2.9 (g) Intermediate Precision

Using Intermediate Precision capturing the variables associated with changes in reagent and calibrator lots, operators, operating conditions. 100 data points will be the optimum number used to express UM. A periodic review of the UM is warranted.

Measurement procedures do not permit components (e.g. sampling/reagent probes, water quality, water-baths, ambient temperature etc.) to be individually studied to ascertain their uncertainty for the combined effect on the variability on measurement results. The QC data used for MU calculations is obtained over a period of time sufficient to capture variability due to routinely occurring changes in the measuring system e.g. reagent and calibrator batch changes, different operators, routine maintenance etc. i.e. intermediate reproducibility or intermediate precision. In measurement procedures that are sufficiently robust that imprecision levels are stable between reagent batches, a combined SD may be used. Adequate data (>100 results) takes longer to obtain for infrequently performed measurement procedures, in which case interim calculations are appropriate, but in any case, including new procedures, a minimum 30 QC results is required before an approximate Gaussian distribution of data points can be reasonably assumed. Thereafter, as QC results accumulate, the imprecision should be regularly re-calculated until the SD is stable at the same number of decimal places used for reported results.

For more on MU calculation please see https://www.westgard.com/hitchhike-mu.htm

2.9 (h) The Difference and Relation Between UM and TE

Though UM (Measurement Uncertainty) and TE (Total Error) are related concepts, the UM does not include the concept of Bias as the assumption is that the True Value cannot be known.

However, the purpose of calculating the UM is not to plan QC strategy but to make the uncertainty known to the users of the lab. As per ISO 15189, upon request the lab has to make the UM available to the users.

- TE provides an approximate worst case value for the error of a measuring system.
- TE is useful for setting upper limits of allowable error.
- MU is not concerned with estimating the total error of a measuring system.
- MU is concerned with estimating an interval of values within which the 'true' value of a measured analyte is believed to lie, with a stated level of confidence.
- MU considers a single measurement result to be the best estimate of a true value, and centers on it the dispersion of other values that could have been obtained if the measurement had been repeated (usually with ~95 % confidence).
- MU is the appropriate approach for meaningfully comparing measurement results with reference values and previous results of the same kind.

In summary, MU does not estimate error, but provides a quantitative estimate of where the true value of a measured analyte is believed by the laboratory to lie, with a stated confidence level. MU is therefore an essential parameter of the reliability of measurement results.

2.9 (i) Reporting Conventions

- 100 mg/dL is the result and if 3 is the SD +/- 3 Defines the result and Combined Standard Uncertainty (μc)
- 100 mg/dL +/- 6 – Defines the result and the expanded uncertainty (U with k=2)
- 100 mg/dL +/- 6 mL at 95% confidence level. – Defines the expanded uncertainty at the specified confidence interval

2.10 Average of Normals (AON) & Bull's Algorithm

AON method is based on the principle that mean value of all normal results fluctuate between well-defined limits. AON method detects only systematic error. This method is mostly used for biochemistry analyzers.

The laboratory collects data for an analyte from a fixed number of healthy persons. Its mean value and standard deviation is calculated. This value will be used as control value.

Bull had determined that some hematological parameters have very small biological variation (CVg) resulting in their mean value remaining steady. Bull applied his idea in erythrocyte indexes (MCV, MCH, MCHC) at the beginning, but today his algorithm is used for the majority of hematological parameters. This is an effective way of determining equipment performances for systematic errors.

In Bull's algorithm a moving average of all tests done is considered as the anchor and determiner of equipment performance instead of a single QC mean value. The moving

average is a mean value that compares with the mean value of a former moving average. In Bull's algorithm the moving average is usually calculated by a batch of 20 values which is compared with the previous means of each 20 values. Bull's moving average is symbolized as XB.

Setting Means

If done manually, the means of MCH, MCV and MCHC are to be calculated from a pool of about 200 normal patients after removing the outliers. This will give robust mean values of the population served. In many automated analyzers, these values maybe factory set and may need modifications as per the averages of the population served. Subsequently when the algorithm is used for monitoring equipment performance, averages of the Bull's algorithm uses all the patient values not only the normal ones.

Interpretation

Bull's algorithm detects only systematic errors and it has its own control chart and its own rules. If Bull's algorithm is used for the quality control of erythrocyte indices the control limits of Bull's chart are B X ± 3%. The range ± 3% comes from the biological variation of the erythrocyte indices which is around 1%. Any shift in calibration will result in shifting averages provided the population served is, within reasonable limits, the same. If either of two criteria are satisfied: (1) the Bull's mean of one of the red blood cell indices is outside its 3% limits, or (2) the average of three consecutive Bull's means is outside its 3% limits, the equipment requires attention. The mean of each batch is compared to Bull's mean and its action limits, i.e. the percent deviation of Bull's mean.

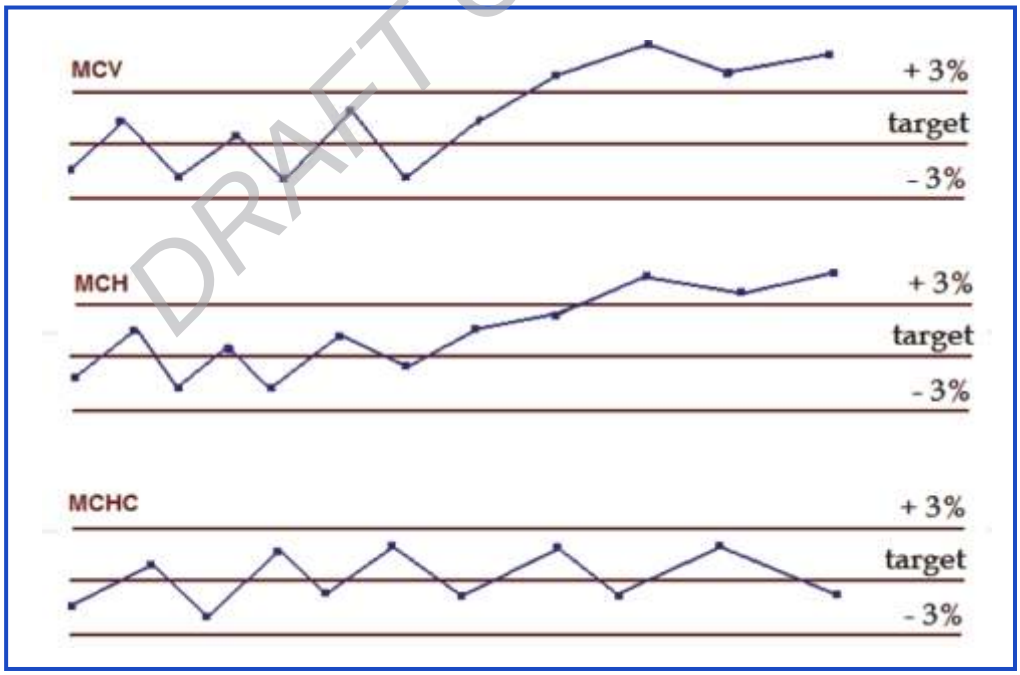


Figure 50: Bull's Algorithm for monitoring stability of CBC counter

In the above example, the MCV and MCH are above 3% of the target. Since both these have, in their calculations RBC count as the denominator, it can be assumed that the RBC count has fallen due to calibration errors. A total of 6 data points have been plotted on the upward trend, pinpointing the time the defect has occurred to about 120 tests earlier (6*20). This will aid the lab in the root cause analysis tremendously.

2.11 Radar/ Spider Charts

A Radar Chart is a graphical method of displaying multivariate data in the form of a two-dimensional chart of three or more quantitative variables represented on axes starting from the same point.

Some CBC analyzers employ radar graph to denote QC and XB data.

The data of QC values from the selected QC file is displayed on the radar chart. If there are no plots it means that no run are available in the selected QC file. Only the outline and parameter names are displayed.

Parameter names are displayed as text on an outer circle. If the latest QC data falls outside the QC limit values it displayed on radar chart.

Inner line	Lower limit value of QC
Outer line	Upper limit value of QC
Blue Central line	Target value
Green irregular line	Latest QC data from the QC file selected in the file list

For points which fall beyond the upper or lower limit, a red "X" is plotted on the upper or lower limit. Data equals the target value: Plotted on the central line. (Blue line in the graph)

Data exceeds the upper limit: Plotted on the upper limit line as a red "X".

Data falls below the lower limit: Plotted on the lower limit line as a red "X".

In the example below, RBC is higher than acceptable and Hb is lower than acceptable and are flagged by red X. Other values are acceptable.

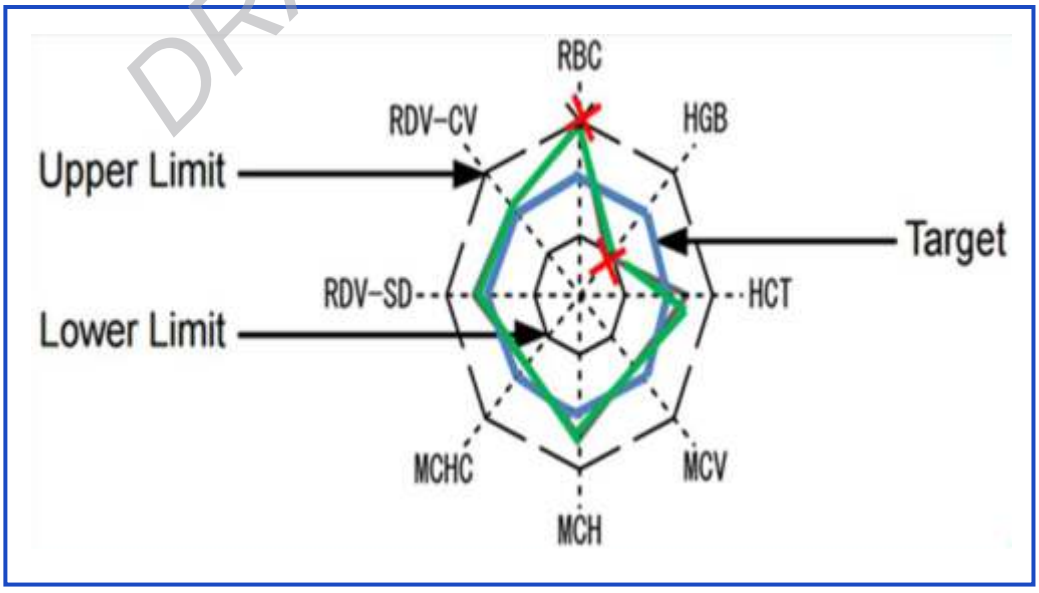


Figure 51: Radar graph used in QC monitoring in Hematology Analyzers

Multiple radar charts may be built in for parameters of QC and XB.

2.12 Harmonization/ Comparability of Tests

Many labs use multiple equipment for the same tests. There could be differences in the performance of each equipment. The traceability of the reference materials would be different. The equipment may even have different method of testing. Thus if the laboratory uses more than one measuring system where the measurements are not traceable to the same reference material / reference method, or the biological reference interval are different, it is essential to perform a comparability study between the systems and prove that there is agreement in performance throughout appropriate clinical intervals. This is recommended at least twice in a year using suitable statistical procedures such as Bland - Altman plot and / or regression analysis. This kind of analysis explained in detail in subsequent sections.

Though not a standard procedure, this exercise may also be employed in ongoing method evaluation in resource limited settings. If one equipment is adequately performance-evaluated on an ongoing basis, this can be used as reference equipment and the other similar equipment compared to this daily. An assessment of the difference percent may be employed to evaluate the performance of the equipment. **Labs for Life QC Tool: Harmonization / Comparability of Tests**

2.13. Conclusion: SQC

The discussions above give details of the Quantitative QC, from the point of purchase through applications in daily monitoring using LJ graphs, assessment of Total Error and Sigma-metrics and being used in planning of ongoing method performance and planning. It also briefly discusses the concept of Measurement Uncertainty and other statistical methods for performance evaluation.

CHAPTER 3: PROFICIENCY TESTING OR EXTERNAL QUALITY ASSURANCE

Learning Objectives

At the end of this chapters, the learners will be able to understand the

- ISO requirements for proficiency testing (Inter-laboratory comparison)
- Different mechanism for proficiency testing
- Assessing acceptability of the proficiency testing reports
- Frequency and scope of testing of some commonly used EQA in India (in annexure)

External Quality Assurance monitor the accuracy of the laboratory's methods on an ongoing basis. It enables the lab to compare itself with others using the same method for the same analyte. Essentially EQA involves use of the same sample in several labs and comparing the lab's results with that of others performing the same test by the same method. Participation in EQA enhances the confidence of the lab in its results. It also enhances the users' confidence in the lab they use for their tests.

3.1 ISO Requirements

Several terms are used interchangeably to denote External Quality Assurance processes.

ISO 15189: 2012, Clause 5.6.3 uses the terms Inter laboratory Comparisons (ILC) and External Quality Assurance (EQA) synonymously. It mandates that the laboratory participate in an ILC/EQA appropriate to the examinations and interpretations of the examination results. It further says the laboratory understand the interpretations of the report and do appropriate corrective actions whenever necessary.

The accreditation standard for ILC/EQA is 17043. The laboratory should strive to participate in an ILC/EQA program accredited by or at least substantially fulfill the relevant requirements of 17043.

ISO also mandates that the ILC/EQA samples should be integrated into the routine laboratory testing process and not be treated as special category. It also requires to be run by the same staff that runs tests. It also says that the ILC/EQA samples should be run just once with no confirmatory run.

Also as per regulatory requirements, the labs are required to keep the raw data of analysis such as equipment printouts of proficiency testing, for verification in audits

Kinds of External Quality Assurance

This module is referring to ILC/ EQA as of 3 of kinds:

- Proficiency testing or PT
- Inter Laboratory Comparison (ILC) program by peer group
- Split testing (by exchange of samples)

3.2 Proficiency Testing or PT

It is the testing of unknown samples from a common pool is sent to a laboratory by an approved PT program provider. Most sets of PT samples are sent to participating laboratories three or more times per year. After testing the PT samples in the same manner as its patient specimens, the laboratory reports its sample results back to their PT program. The program grades the results using some approved grading criteria and sends the laboratory scores reflecting how accurately it performed the testing.

3.2.1 PT Report Attributes

The following are the requirements that should be seen on PT reports

A. Analyte name
B. Units of reporting of a parameter
C. Survey sample ID
D. Reported results
E. N: the number of participating labs.
F. Mean
G. Expected Range
H. SD
I. SDI/Z score/other parameters for comparison
J. Grade/ Acceptability

The following figure shows an example. Analyte name, units of reporting, sample IDs, reported results and N are circled for ease of understanding

Hematology Analyte/Method	Sample	Reported Result	N	Expected Result	Mean	SD	SDI	Grade
Platelet Count (x10E9/L)	XE-06	61	36	43 - 72	57.4	2.8	1.3	Acceptabl
Sysmex XE-2100	XE-07	489	30	363 - 606	484.4	11.0	0.4	Acceptabl
	XE-08	336	31	250 - 418	333.7	8.2	0.3	Acceptabl
	XE-09	113	31	87 – 147	117.3	4.7	-0.9	Acceptabl
	XE-10	209	33	158 - 265	211.8	7.0	-0.4	Acceptabl

Figure 52: Attributes of PT/EQA reports (1)

The above example shows a PT result for platelets. Please note the unit definition. In India, platelets are generally expressed in Lakhs or Hundred Thousands (103) /uL.

In this example, one PT includes 5 levels to include several clinical decision levels. Reported results and N are given.

The N

The N is the number of participating labs in the PT program. The providers may define and compare (explained later) the participant lab's result in several ways.

If N is too low, statistics may not be calculated for that peer group by the PT provider. The higher the N, the better an estimate of the target value can be determined for that PT sample. The higher the N, the more data points can be used to calculate the SD for the group. The higher the N, the less impact aberrant results or incorrectly defined outliers will have on the group's SD and/or mean. At the least lowest minimum N of 10 is required. An N of 100 gives very good anchoring of the mean. A 30 in is an acceptable good number.

- as part of all reports submitted for that analyte or;
- more specifically as part of the reports performed by the same method for that anlayte or;
- most specifically, by the same method and equipment as the participant lab, for that analyte;
- N is equally important in all the above scenarios

3.2.2 Acceptance Criteria

Hematology Analyte/Method	Sample	Reported Result	N	Expected Result	Mean	SD	SDI	Grade
Platelet Count (x10E9/L)	XE-06	61	36	43 - 72	57.4	2.8	1.3	Acceptable
Sysmex XE-2100	XE-07	489	30	363 - 606	484.4	11.0	0.4	Acceptable
	XE-08	336	31	250 - 418	333.7	8.2	0.3	Acceptable
	XE-09	113	31	87 – 147	117.3	4.7	-0.9	Acceptable
	XE-10	209	33	158 - 265	211.8	7.0	-0.4	Acceptable

For CLIA, the $\%TE_A$ for platelets is 25%

TE_A in units = 57.4 * 0.25 = 14.4

Lower Limit = 57.4 – 14.4 = 43

Upper limit = 57.4 + 14.4 = 71.8 rounded to 72

Figure 53: Attributes of EQA reports (2)

The Mean and the Range

The above table shows the range of the expected results as well as mean of all the participants. The mean is the average results of all participants, after removing the outliers. In a PT, the mean of all inliers is the best estimate of the true or **expected value.**

Range can be determined by one of three ways

1) Target Value ± specified value (as in the above example, the specified value being the TE_A in units, at the target)
2) Percentage ± specified %
3) Multiple of PT group standard deviation (SD) ± specified 2 or 3 Sds

The range is calculated in the above example by deriving the TE_A at mean and adding and subtracting it from the mean. Acceptable performance criteria are defined by the service provider and should be understood by the participant lab. The service provider is required to tell the users the mechanism of assigning the acceptable range.

If 80% of the submitted results do not fall within the limits of acceptability, then no results are graded.

3.2.3 Scoring Systems

This section explains a few scoring systems used by PT providers. A list of PT used in India is also simultaneously incorporated as per their reporting system. A guideline on each provider's scope and sample frequency is also attempted. For more details, the reader is advised to visit the corresponding sites.

3.2.3 (a) SDI (Standard Deviation Index) / Z score

The data from all the laboratories are usually analyzed to determine an overall average and standard deviation for the group. The program will generally report your performance relative to the group. The difference between your test results and the overall average is often expressed by a standard deviation index, or SDI, which expresses the difference in terms of the number of standard deviations from the overall mean. Thus SDI/Z-score is a calculated value that tells how many standard deviations the reported value is from the expected value for that material. It is calculated by taking the difference between the reported value and the expected value, then dividing by the standard deviation observed for that control material from the analyses in all participant labs. For example, for reported value of 112 for an expected value of 100 and a standard deviation of 5, the SDI/Z-score is 2.4 [(112- 100)/5] denoting 2.4 standard deviation in the positive direction from its expected value

On a series of specimens, if you observe SDIs such as +1.5, +0.8, +2.0, +1.4, and +1.0 (all positive), this suggests that your method is generally running on the high side and is biased, on average, by +1.3 SDI.

Difference between SDI and the Z-score.

They're basically the same thing, but the Z-score tends to be used more in Internal QC programs to compare an individual QC result with the expected values for that material, whereas the SDI tends to be used in external QC programs to compare the performance of the lab with the overall mean for a defined comparative group or with an established target value.

SDIs allow you to inspect results from many different tests at the same time, without having to think about different units and the actual magnitude of the change in the units of the test. In general, any SDI of 2.0 or greater deserves some special concern, regardless what the test is. Any test whose average SDI is 1.0 or greater deserves some special attention because your method shows a systematic difference from the group. In the future, this bias might lead to unacceptable results.

Hematology Analyte/Method	Sample	Reported Result	N	Expected Result	Mean	SD	SDI	Grade
Platelet Count (x10E9/L)	XE-06	61	36	43 - 72	57.4	2.8	1.3	Acceptable
Sysmex XE-2100	XE-07	489	30	363 - 606	484.4	11.0	0.4	Acceptable
	XE-08	336	31	250 - 418	333.7	8.2	0.3	Acceptable
	XE-09	113	31	87 – 147	117.3	4.7	-0.9	Acceptable
	XE-10	209	33	158 - 265	211.8	7.0	-0.4	Acceptable

Figure 54: Attributes of EQA reports (3)

Please note that SDI/Z score has both value and direction, indicating that it can be a positive or negative value.

3.2.3 (b) Residual

In some EQA Schemes like NARI (National AIDS Research Institution), the difference between the Target Value and the Observed value is termed as “Residual”. Further calculations for SDI is as above.

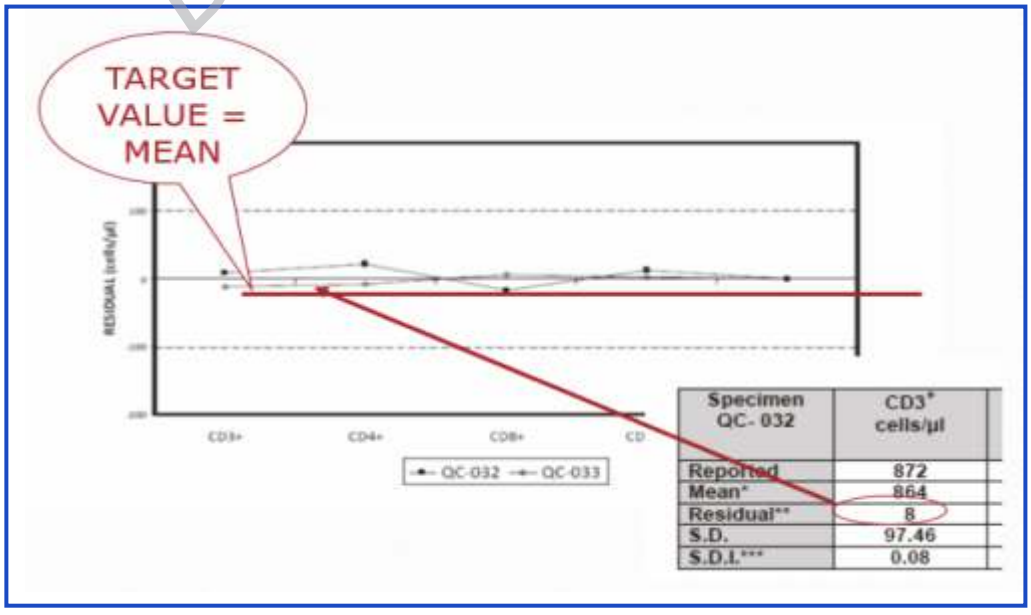


Figure 55: Residual, NARI EQAS

3.2.3 (c) Histograms and Line Graphs

The example below (Figures from 56 to 58) takes the reader through a chemistry PT report. The outer page has a flag warning that an analyte, Total T4, has a Z (SDI) score of between 2 and 3. (Figure 56)

The same report on the inside page, among other analyte reports says Total T4 has a reported result of 14.2 whereas the expected result is 12.3. This is a borderline outlier as per the service provider's criteria of acceptance, eliciting a Z score of +2.77 (Figure 58). The Running Mean Z score (RMZ) average of SDI or mean Z score is the average of all the samples in the cycle for the analyte.

Figure 57 shows the details of the Lab's Total T4 Performance over 12 months as bar graphs (Histograms) and Line graphs (LJ like Plot as well as Yundt Plot ; explained later)

The histograms indicate the performance in the current sample, whereas the line graphs indicate the performance in the past 12 months.

Please note that the lab has been showing a consistent positive bias for the past 8 months. (Graph in the green circle, with months on the X axis and Z scores on the Y axis).

Below that is another line chart, Yundt Chart (Blue circle with Expected values on the X axis and Z score on the Y axis). This shows more positive bias points at higher concentrations. Both these graphs together tell you about a shift in accuracy towards in the positive direction over a period of months, especially in higher concentrations of the analyte.

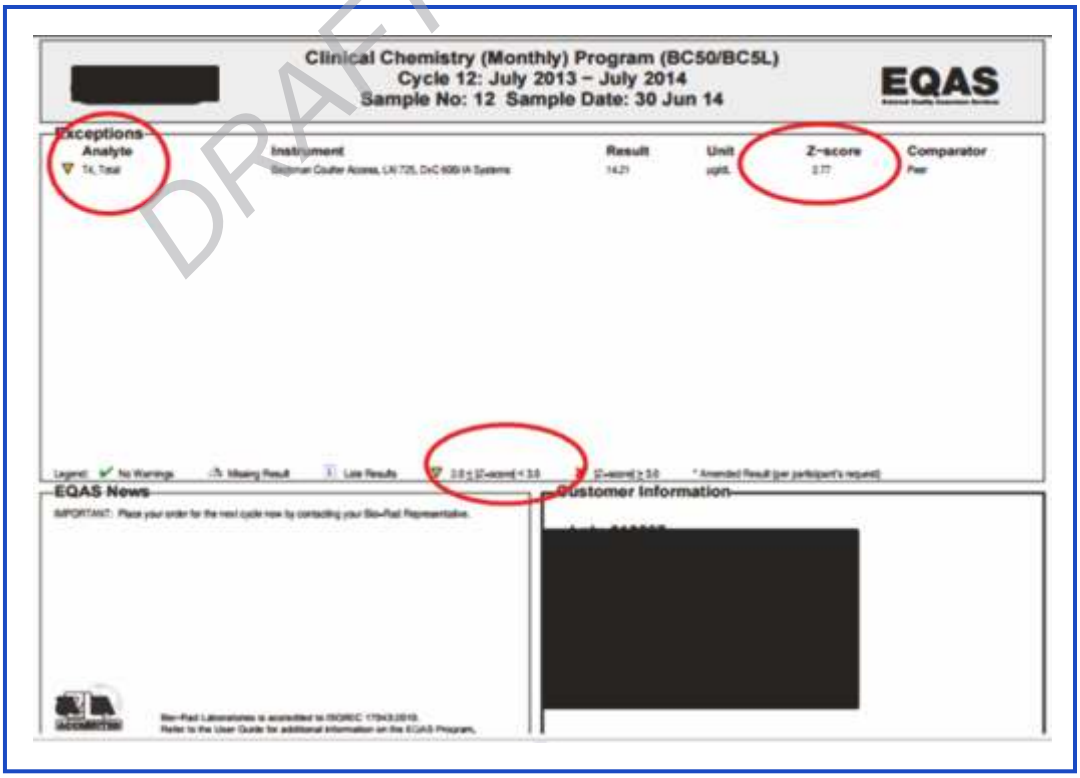


Figure 56: Page 1 of Biochemistry EQA, flagging a warning in T4 total

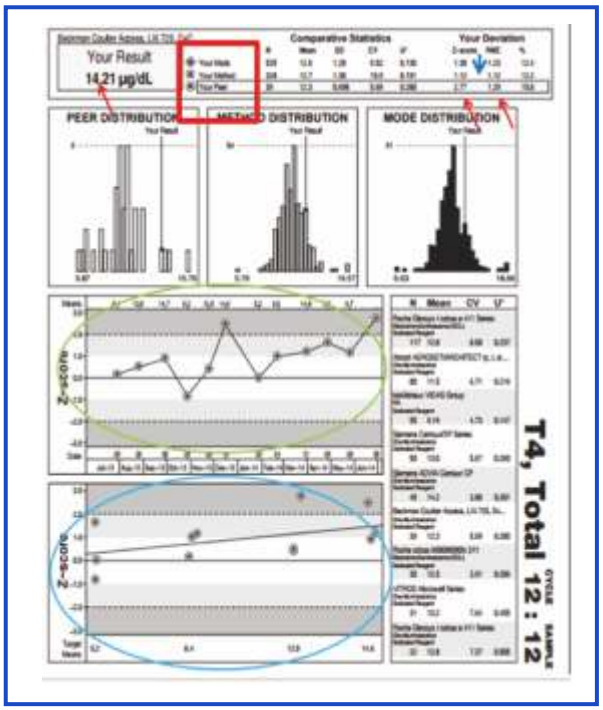


Figure 57: Summary page showing all analytes with details of ranges and SDs, Z scores and RMZ etc.

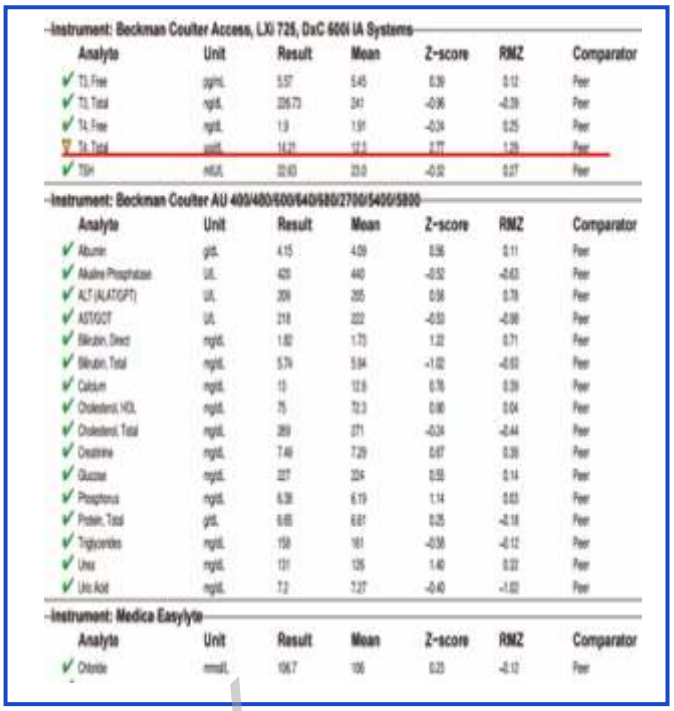


Figure 58: The details of T4 in the last 12 cycles showing patterns of bias in the upper level in Yundt plot

The Comparator

To continue with the above figure, see the red box which shows 3 comparators

- The current month's report of 14.21 has a +2.77 Z score in peer group comparisons (Red Arrows)
- There are 2 more comparison groups (Blue Arrow); "Your Mode and" "Your Method", the first denoting all the results obtained, in this case for Total T4 in that cycle (with N of 535) and the second, all the Total T4 results done by CLIA method but on many different equipment (N of 328), and the third your peer (same method, same equipment), with an N of 39. Whereas the first 2 comparisons show a better Z score, the third the more specific peer comparison shows a higher Z score. Please note how the N reduces as the comparison mode becomes specific. However, 39 is still a robust N. These numbers must be taken into consideration as you evaluate your PT report
- Quick reference bar graphs as histograms thus give visual assistance to the lab's performance with reference to the comparators.

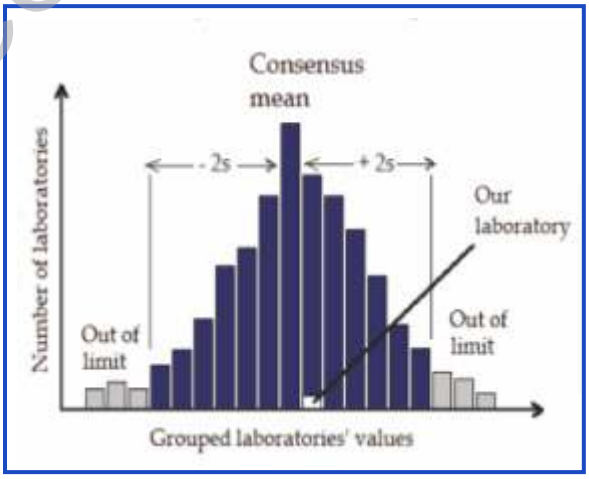
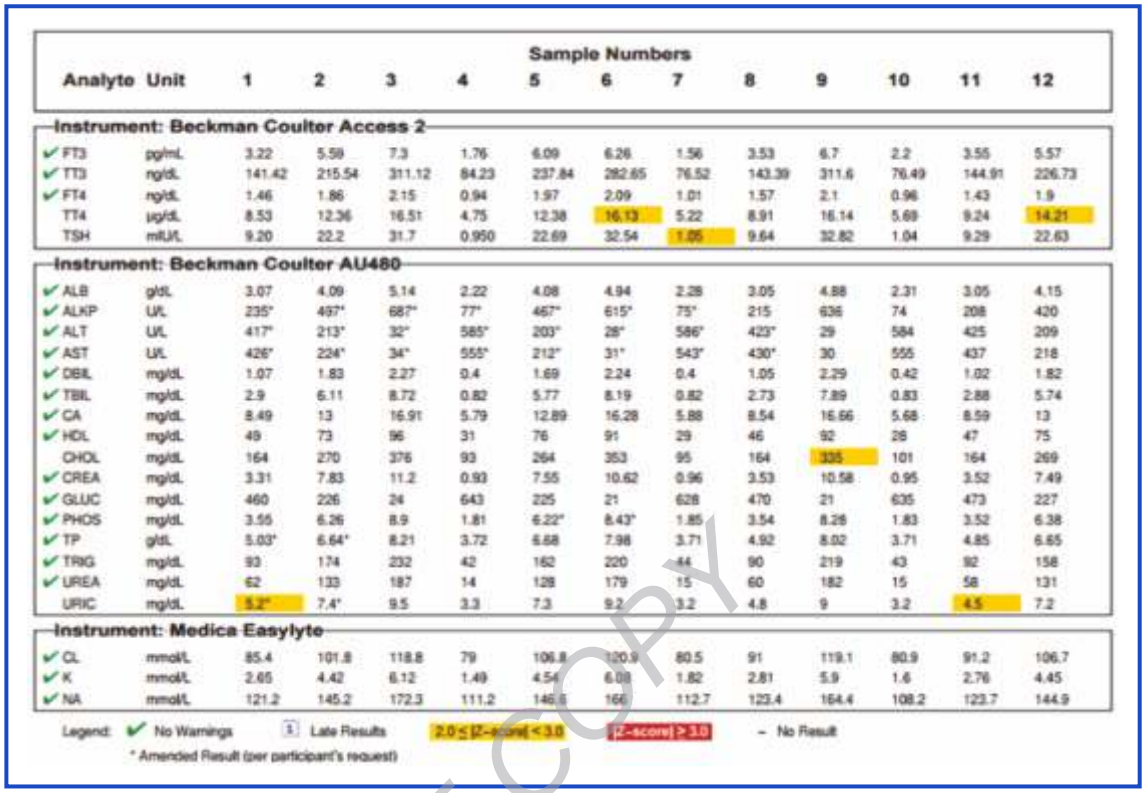


Figure 59: Histograms with consensus mean & limits

Thus the best indicator of accuracy can be obtained by comparing with the same method and equipment. The lab should understand the comparator in the program it is participating in

3.2.3 (d) Cumulative Reports

Cumulative reports will also be made available by some providers giving a quick overview of the long term, comprehensive performance of the lab, analyte wise. See below.



Analyte	Unit	Sample Numbers 1	2	3	4	5	6	7	8	9	10	11	12
Instrument: Beckman Coulter Access 2													
✔ FT3	pg/mL	3.22	5.59	7.3	1.76	6.09	6.26	1.56	3.53	6.7	2.2	3.55	5.57
✔ TT3	ng/dL	141.42	215.54	311.12	84.23	237.84	282.65	76.52	143.39	311.6	76.49	144.91	226.73
✔ FT4	ng/dL	1.46	1.86	2.15	0.94	1.97	2.09	1.01	1.57	2.1	0.96	1.43	1.9
TT4	µg/dL	8.53	12.36	16.51	4.75	12.38	16.13	5.22	8.91	16.14	5.69	9.24	14.21
TSH	mIU/L	9.20	22.2	31.7	0.950	22.69	32.54	1.05	9.64	32.82	1.04	9.29	22.63
Instrument: Beckman Coulter AU480													
✔ ALB	g/dL	3.07	4.09	5.14	2.22	4.08	4.94	2.28	3.05	4.88	2.31	3.05	4.15
✔ ALKP	U/L	235*	497*	687*	77*	467*	615*	75*	215	636	74	208	420
✔ ALT	U/L	417*	213*	32*	585*	203*	28*	586*	423*	29	584	425	209
✔ AST	U/L	426*	224*	34*	555*	212*	31*	543*	430*	30	555	437	218
✔ DBIL	mg/dL	1.07	1.83	2.27	0.4	1.69	2.24	0.4	1.05	2.29	0.42	1.02	1.82
✔ TBIL	mg/dL	2.9	6.11	8.72	0.82	5.77	8.19	0.82	2.73	7.89	0.83	2.88	5.74
✔ CA	mg/dL	8.49	13	16.91	5.79	12.89	16.28	5.88	8.54	16.66	5.68	8.59	13
✔ HDL	mg/dL	49	73	96	31	76	91	29	46	92	28	47	75
CHOL	mg/dL	164	270	376	93	264	353	95	164	335	101	164	269
✔ CREA	mg/dL	3.31	7.83	11.2	0.93	7.55	10.62	0.96	3.53	10.58	0.95	3.52	7.49
✔ GLUC	mg/dL	460	226	24	643	225	21	628	470	21	635	473	227
✔ PHOS	mg/dL	3.55	6.26	8.9	1.81	6.22*	8.43*	1.85	3.54	8.26	1.83	3.52	6.38
✔ TP	g/dL	5.03*	6.64*	8.21	3.72	6.68	7.98	3.71	4.92	8.02	3.71	4.85	6.65
✔ TRIG	mg/dL	93	174	232	42	162	220	44	90	219	43	92	158
✔ UREA	mg/dL	62	133	187	14	128	179	15	60	182	15	58	131
URIC	mg/dL	5.2*	7.4*	9.5	3.3	7.3	9.2	3.2	4.8	9	3.2	4.5	7.2
Instrument: Medica Easylyte													
✔ CL	mmol/L	85.4	101.8	118.8	79	106.8	120.9	80.5	91	119.1	80.9	91.2	106.7
✔ K	mmol/L	2.65	4.42	6.12	1.49	4.54	6.08	1.82	2.81	5.9	1.6	2.76	4.45
✔ NA	mmol/L	121.2	145.2	172.3	111.2	146.8	166	112.7	123.4	164.4	108.2	123.7	144.9

Legend: ✔ No Warnings 1 Late Results 2.0 ≤ \|Z-score\| < 3.0 \|Z-score\| ≥ 3.0 – No Result

* Amended Result (per participant's request)

Figure 60: Cumulative reports: EQAS

3.2.3 (e) The Target Score (TS) and %Deviation by Concentration Chart

TS allows participants to assess their performance at a glance.

The TS relates the %Deviation of the reported result from the Mean to a Target Deviation for Performance Assessment (TDPA). TDPAs are set to encourage participants to achieve and maintain acceptable performance. TDPAs are fit-for-purpose performance criteria which are set taking guidance from ISO/IEC17043, ISO13528 and IUPAC. Target

Deviations for Performance Assessment are also used to calculate the Standard Deviation for Performance Assessment (SDPA).

%Deviation by Concentration Chart is similar to the Yundt plot and enables rapid assessment of concentration related biases. Biases at low or high concentrations can be easily determined

3.2.3 (f) The Target Score (TS) Plot

%Deviation by Concentration Chart

This is in principle similar to the Yundt plot, with concentrations on the X axis and % Deviation on the Y axis, allowing the lab to understand biases at specific concentrations to enable calibrations and correction of the bias.

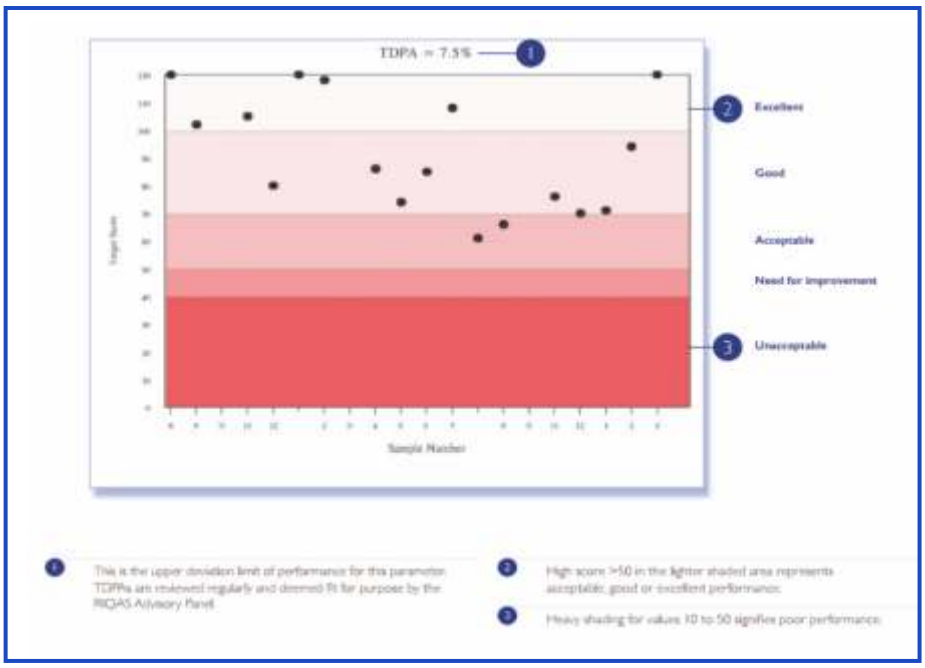


Figure 61: Target Deviation for Performance Management

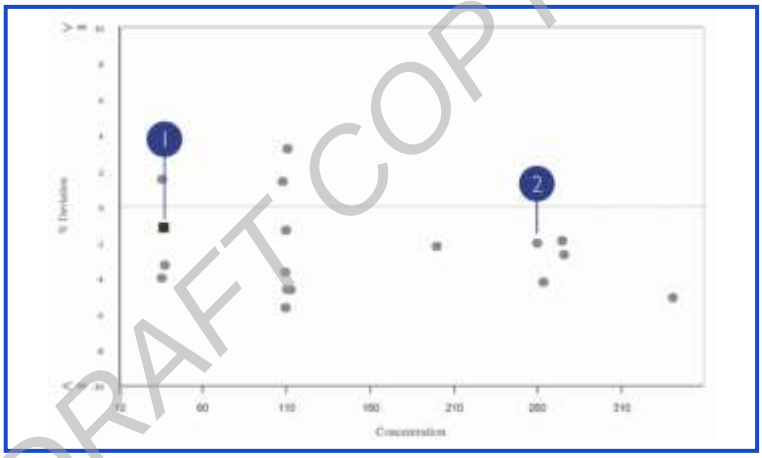


Figure 62: Percent deviation plot

3.2.3 (g) The VIS Scoring

The VIS or Variance Index Scoring is another scoring system used in India, especially in CMC Vellore Biochemistry EQAS.

The VIS system was first proposed by the United Kingdom National Quality Control Scheme (UKNEQUAS). It uses CCV (Chosen Co-efficient of Variation) & DV (Designated Value/ Expected value) used to calculate VIS, CCV being the Allowable Limit of Error for an analyte (TE_A) (Please see Table below), the sum of both imprecision and bias. This method has been set & recommended by WHO after studying the performance of many Indian labs.

The calculation is done in 2 steps.

1) % Variation [%V] = {(Reported Value – Expected value)/ Expected Value} *100

2) Variance Index = (% V/ CCV) X 100

Example: If in a Glucose EQAS cycle, the Expected Value is 120 mg % and Reported Value is 95 mg%,

1) % Variation [%V] = {(120-95)/ 120} X 100 = 20.8
2) VIS = (20.8/7.5)* 100 = 277

Lower the VIS, better the lab's accuracy. Ideally the VIS should be less than 100. The CMC scores all VIS < 50 as zero score. Any score >400, it is given as 400. Any VIS score >150 requires investigation and corrective action.

VIS Score Interpretation

- < 100 Very good
- 100 -150 good
- 150 -200 satisfactory room for improvement
- > 200 Not acceptable

In the CMC Biochemistry EQAS, 'Designated Value' is the value obtained after excluding results, from labs with same method, which are > 3SD of Method Mean and recalculating the mean after eliminating the outliers: Mean of 'inliers only'

CCV of some common Analytes

Glucose	7.5	Sodium	2.3
Urea	10	Potassium	5.0
Creatinine	10	Chloride	6.0
CK	7.3	AST	12.5
T.Bilirubin	19.2	ALT	17.3
T.Protein	7.5	ALP	15.5
Albumin	7.5	Amylase	15.5
Calcium	6.0	LDH	15.5
Uric acid	7.7	Phosphorus	7.8
Cholesterol	7.5	Bicarbonate	9.0
TGL	14	HDL- C	7.6
HDL	7.6	Iron	15

Figure 63: CCV of common analytes

Another term seen on the reports is VCRM (Value corrected to the Reference Mean). This is the mean obtained at the organizing lab after exposing the QC samples to ambient temperature (25-35 C) for a period of 7- days (transport time) and analyzing them on five different days. This is to factor in the difficulty in sample transportation in difficult terrains.

In addition the CMC EQA gives mechanisms of assessment of performance such as:

SDI = (Reported value- Expected Value)/Group SD

% Bias = (Reported value- Expected Value)/Expected Value *100

3.2.3 (h) Z Scoring Within and Among Labs

AIIMS CBC and Peripheral smear EQA requires 2 runs of the CBC sample. The report includes the Z score among labs for assessment of accuracy and Z score within lab for an idea of precision. The acceptance report also thus has 2 components to it, Among Lab EQA and Within Lab IQA. The qualitative reporting on peripheral smear is graded as satisfactory or unsatisfactory.

3.2.3 (i) Youden Plots

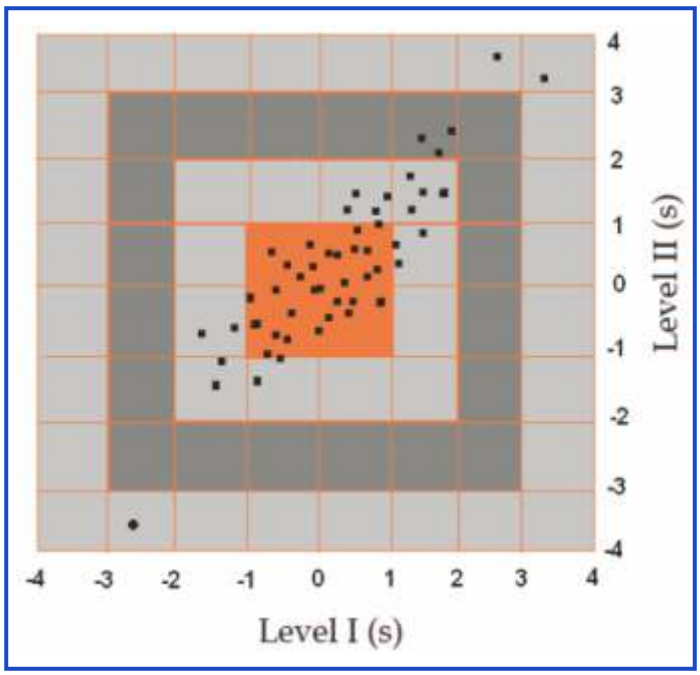


Figure 64: Youden Plot

Some EQA schemes use two control samples of different levels in order to check the performance of the analytical method in different concentration/activities, and preferably close to the decision limits. When 2 EQA samples – high and low are analysed by each lab either by same method analytical principle, instrument, reagent (or by different techniques in which case a different plot is made), the observed results plotted as a Youden plot. This allows comparison of the relationship of each level's value to the group's performance. Youden plot is a rectangular chart of which the four angles correspond to the control limits of the two control levels [-4SD - +4SD]. The acceptable part, the mid-zone and the rejected part have different colors. Each dot represents a different laboratory and therefore Youden plot describes the whole EQAS scheme. Dots (laboratories) that lie across the diagonal of the rectangular, at 45o (The Manhattan Mean or MM), but are far from the center correspond to laboratories with proportional analytical error. The greater the distance from the center, the greater the proportional error. Dots restricted in the central rectangular, correspond to laboratories of which the performance is considered acceptable for this specific analyte. The service providers mark your lab among the dots.

In India, The CMC Hemostasis EQAS adopts this scheme in addition to the bar graphs. Both peer and method comparisons are made and the acceptability reported as within/ Out-with consensus.

3.3 Qualitative and Semi-Quantitative EQA in India

Qualitative assessment of tests are done for staining, culture and serology in microbiology, cytology, histopathology, IHC, Peripheral smears in pathology, blood grouping, cross matching, Coomb's testing and TTIs in blood banking are available with many service providers.

Please see the annexure no 5 for the frequency and Scope of Testing of Commonly used EQA Schemes in India

3.4 Inter Laboratory Comparison (ILC) Programs (Peer Group Comparisons)

Several IQC providers, make available ILC data. These are robust checkpoints for the evaluation of accuracy of the lab tests. For several reasons, the peer group comparison data may be considered as the most robust version of ILC/EQA.

The ILC peer groups are created by the service provider of the lab's Internal Quality Controls and thus is it is very important to build the peer group data availability into the IQC purchase. The service providers creates groups for the same method and equipment as well as that for all labs reporting on that analyte; often termed group values, on an ongoing basis. The labs feed their data into the centralized base. The lab's results may then be compared with the peer group for both accuracy and precision and the reports made available periodically.

- Your laboratory enrolls in an inter-laboratory program offered by your QC manufacturer.
- Your laboratory, along with other laboratories, analyze the same lot number of control materials for the month.
- Your laboratory submits your QC results through a QC data management program to a central facility.
- The central facility examines the data for outliers and calculates the means and SDs for the peer group and all-lab group, and SDI and CVI for your laboratory.
- Your laboratory receives a report indicating your analytical performance.

Consensus Based Metrics such as SDI for accuracy and CVI for comparison of your lab's precision to the other participant labs is also provided. SDI has been discussed in the earlier section. CVI is the Coefficient of Variation Index and is calculated by dividing the lab's monthly CV by the CV of all the values. Ideally, CVI $\leqslant$ 1.0, since your values are from a single lab, while the peer CV is from several laboratories. The smaller the number, higher the precision in your lab.

If CVI = 1.5 to 2.0, your lab is 50-100% less precise than its peer group, usually requiring investigation.

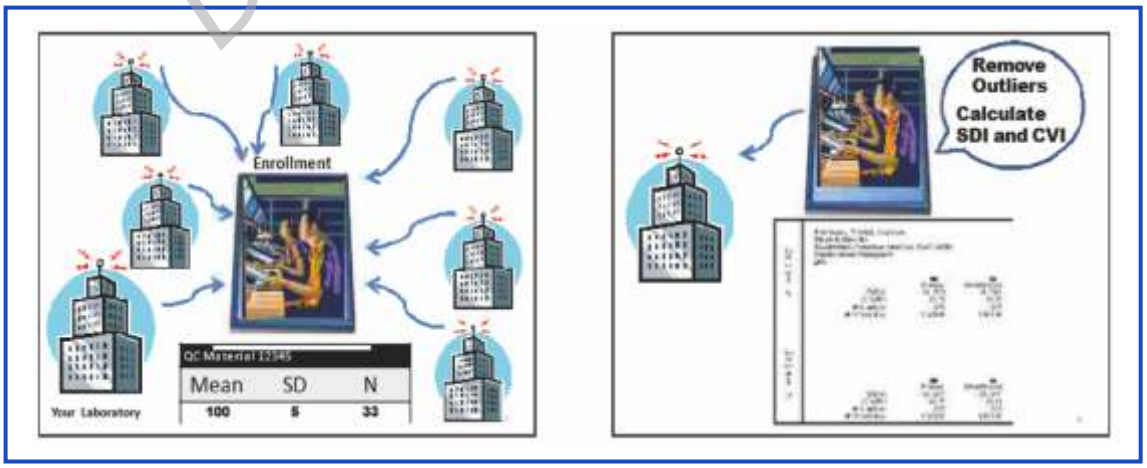


Figure 65 : Diagrammatic representation of collecting, compiling, analysis and dissemination of peer group data

As in the Proficiency Testing Comparators, the ILC groups can be your peer using the same method and equipment (blue arrow) or that of the all-labs group (red arrow). Also monthly as well as cumulative data is made available. (Figure 66)

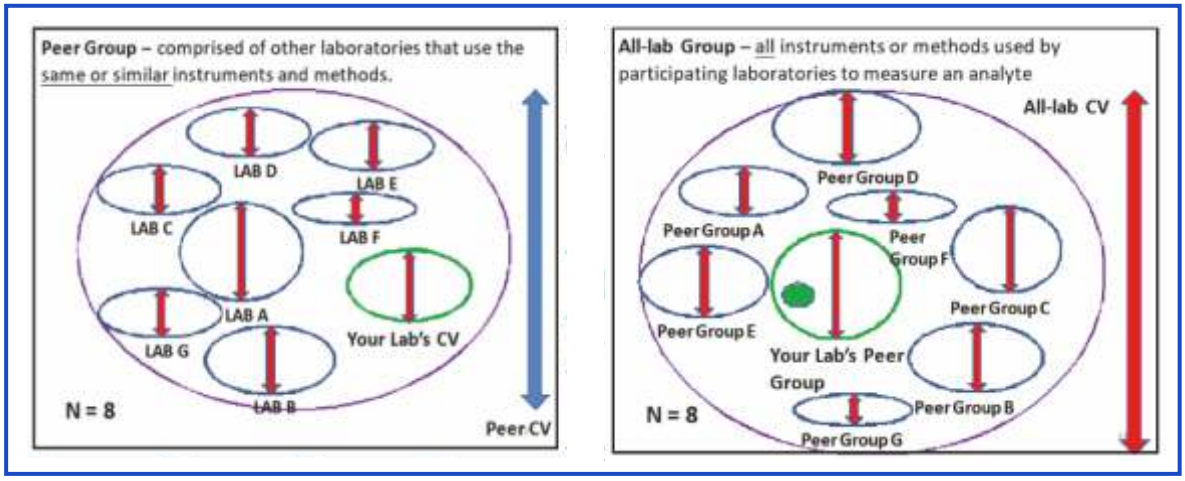


Figure 66: Kinds of peer group comparisons made available in a peer group reports

The figure 67 below shows an ILC report for Direct Bilirubin by diazotization method (red horizontal circle), done on Beckman Coulter Equipment AU 400 to 5800 (red horizontal circle), by 153 labs collecting 22,609 data points (L1 & L2, Cumulative). Level 1 and 2 controls (Red and Green vertical circles) are used. Monthly and cumulative data (Red and Green dotted arrows) are collected and computed. Mean, SD CV, number of data points and number of labs are shown in the report.

Such a robust mean allows anchoring as the true/ target value for any kind of comparison and calculation. Please also refer to the advantages of having such a target value in the IQC monitoring, enabling the calculation of TE, SEc and Sigma-metrics.

ISO also allows this kind of comparison as an alternative approach albeit in the absence of formal EQA/ILC programs. 5.6.3. 2 last sub clause.

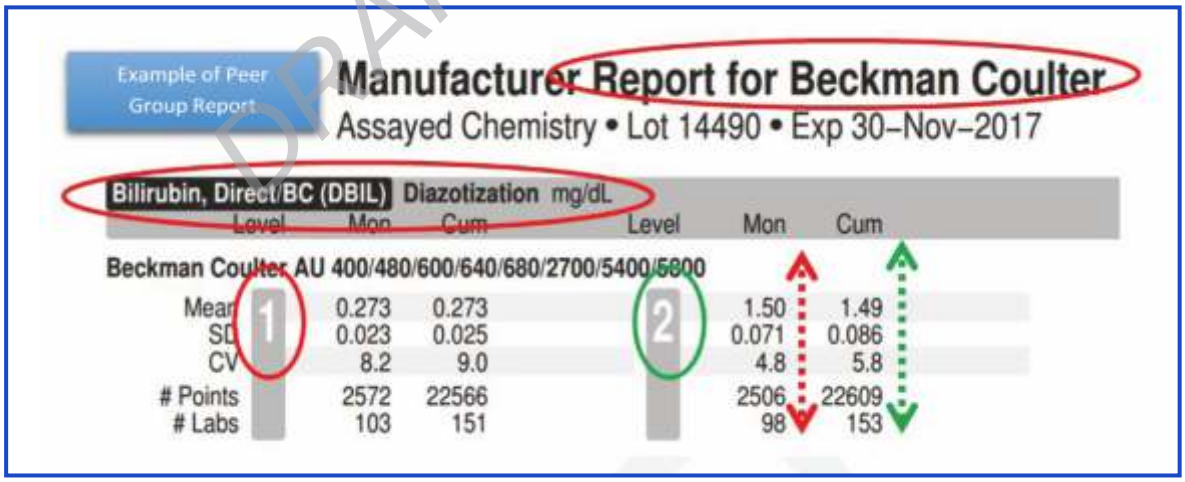


Figure 67: Example of peer group comparison data, specific for equipment and method, for 2 levels of QCs with monthly and cumulative statistics and the number of participating labs and data points

3.5 Split Testing (Exchange of Samples)

For those tests where no formal PT program is available, ISO recommends "exchange of samples with other laboratories" as an alternate method. 5.6.3.2. What is implied here is that the lab send a sample to one or more reliable/accredited labs and compare the results. Using a minimum of 2 comparison labs is recommended. Clinical Pathology samples like Urine,

Stool, and Cavity Fluids are generally analyzed for proficiency in this way. Some high end tests such as Bone Marrow, IHCs, molecular biology and cytogenetics are also subjected to proficiency testing thus. Some of these are available in the international EQAS and must be ideally registered with those providers.

Some labs also do inter-observer variance as a substitute to exchanging samples with other laboratories. For unstable analytes like semen analysis where time lapse affects the motility, such measures may be acceptable. Decisions on these may be taken and documented by the lab in alignment with the requirement of any accreditation bodies.

Periodicity of testing, acceptance criteria, authority for review of acceptance should be defined for each analyte and documented.

3.6 Troubleshooting and Corrective Actions

The following are points to be noted and about wrong PT/EQA reports

Spurious errors should be avoided. As EQAS is appraising the analytical part of the testing, all effort should be directed at avoiding careless mistakes which will result in meaningless EQAS reports

1. Incorrect classification of testing methods leading the service provider to analyze the lab's report with the wrong peer
2. Incorrect units / conversion leading the service provider classify the reports as incorrect
3. Incorrect sample tested. If there is a serial number / lot number in the lyophilized testing material caution must be exercised in identifying the sample correctly
4. Technical errors – reconstitution/dilution inadequate mixing. Have a separate calibrated pipette to do the reconstitution of EQAS samples. A fixed volume pipette will be appropriate.
5. Transcription errors

Please refer annexure 6B for EQA (PT failure checklist) corrective action format

Actual Analytical Errors should immediately lead to serious investigations and root cause analysis.

a. Relook at the IQC data
b. Are there trends? High/low bias?
c. Change in reagents?
d. Changes in calibrators?
e. Look for acceptance testing details, lot verifications.
f. Storage of reagents, Calibrators?
g. Change in the environment?
h. Water quality?
I Operator?
j. Investigate Equipment performance: aspiration system, incubators, cuvette systems, optical system, refrigeration system

PART 2

METHOD EVALUATION

AS PER ISO: 15189 5.3.1.2 AND 5.5.1

CHAPTER 4: METHOD EVALUATION

Learning Objectives

At the end of this chapter, the learners will be able to understand the

- Difference between validation and verification
- Pre-purchase assessment of equipment using statistical tools
- Setting up of acceptance testing program for newly procured equipment

4.1 Validation and Verification

Many a times the terms are used interchangeably. However, they are not the same. Validation is "the process of testing a measurement procedure to assess its performance and determine whether that performance is acceptable" and is typically a manufacturer's activity. Verification is simply verifying the manufacturer's claims for performance specifications. It is typically performed in a clinical laboratory for implementing an FDA-approved instrument/method. It is a much simpler and streamlined method than validation.

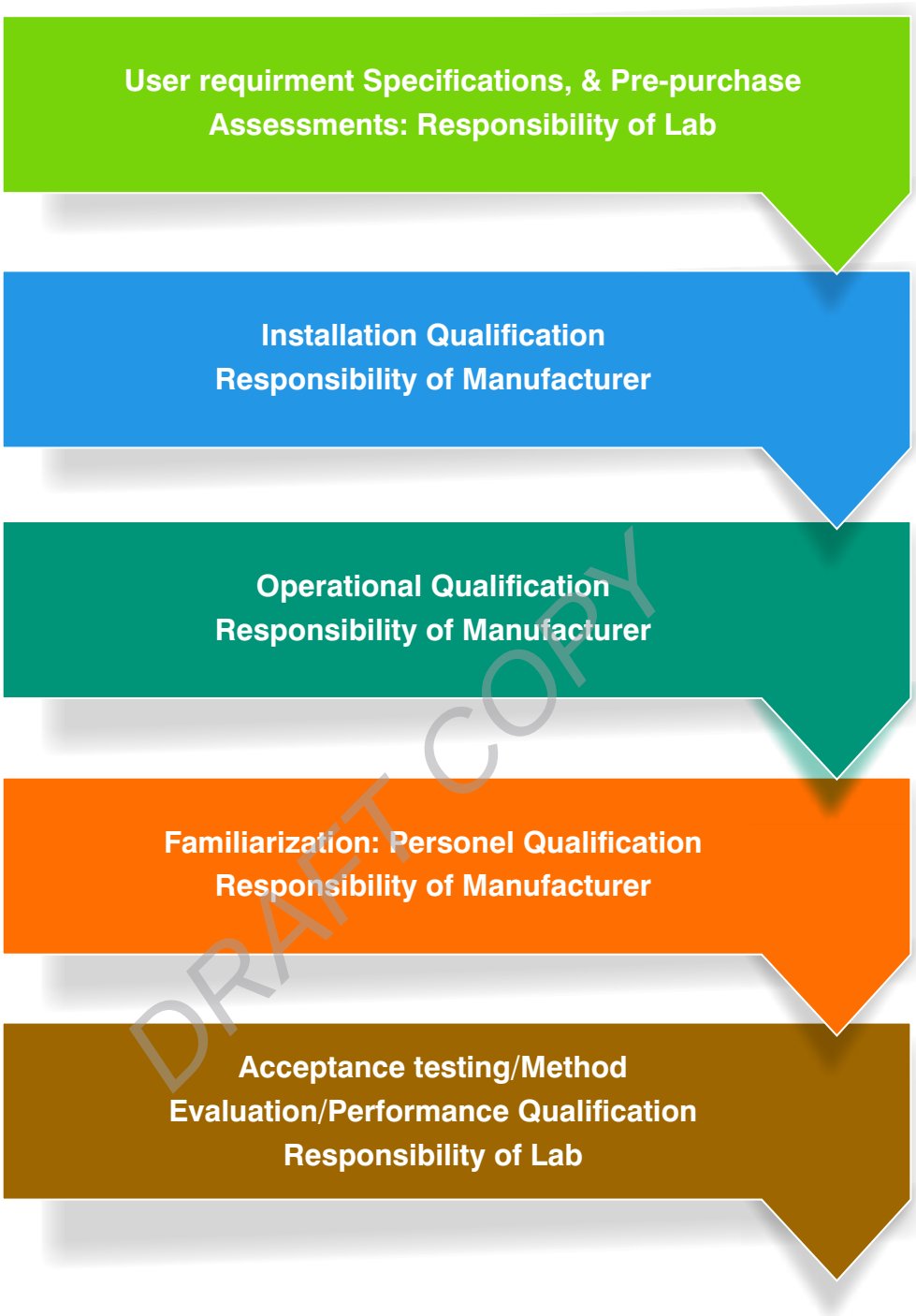
ISO 15189: 2012, in clause 5.3.1.2 mandates equipment acceptance testing. Performance specifications as claimed by the manufacturer is derived under ideal conditions. The working condition of the lab may not be able to replicate that ideal condition. Besides, the transportation of the equipment can affect the factory settings. Thus it is incumbent on the laboratory that upon installation, the equipment is verified and the claims of the manufacturer reestablished.

As per 5.5.1 it is also incumbent upon the laboratory to use validated examination procedures. These procedures are also to be subjected to independent verification in the lab by obtaining objective evidence in the form of performance characteristics, to establish the claims put forward by the manufacturer.

Acceptance testing may further be modified into performance evaluation for fitness of purpose, by using sigma metrics.

This section explains the process of this verification and performance evaluation. In addition, the section explains a method for assessing the "fitness for purpose" of the equipment prior to purchase, by calculating the sigma metrics, using the manufacturer supplied performance data. An FDA approved method just means that the claimed performance specifications have been verified. It does not necessarily mean that the method performance will be acceptable for the purpose for which it is intended. The onus is on the lab to understand this and pre-verify the suitability of the method.

4.2 Process for Introducing a New Method



This module will discuss 2 aspects where the laboratory's responsibility dwells primarily.

1) Pre purchase assessment of methods or equipment
2) Acceptance Testing

*Please refer to the **Equipment Management Module of Labs for Life** for details of the other aspects.*

4.3 Pre-purchase Assessment

When a lab decides to introduce a new test or procure equipment, several factors are considered. An URS or User Requirement Specifications based on the lab's requirement in terms of quality specifications, robustness of the method, cost implications and in the case of an equipment, its throughput, and accessories required, service and spare-part availability, environmental requirements are considered among several others. Based on the URS, the lab may evaluate several brands available in the market. The lab is generally given the product details in the form of product inserts. These inserts will specify the Performance Characteristics. In the case of FDA approved methods, it ensures that these specifications have been verified by the authorities concerned. But what it does not guarantee is the suitability of the method for the intended use. The lab is well advised thus to evaluate the fitness for purpose through evaluation of total error and sigma metrics of the method at all clinical decision levels. This may be done using the 4 key numbers, the CVs or SDs specified by manufacturer, bias values derived from the Slope and the intercept (also supplied by the manufacturer) and TE_A (from any source like BV).

Let us consider two examples, calcium and glucose, from certain product inserts

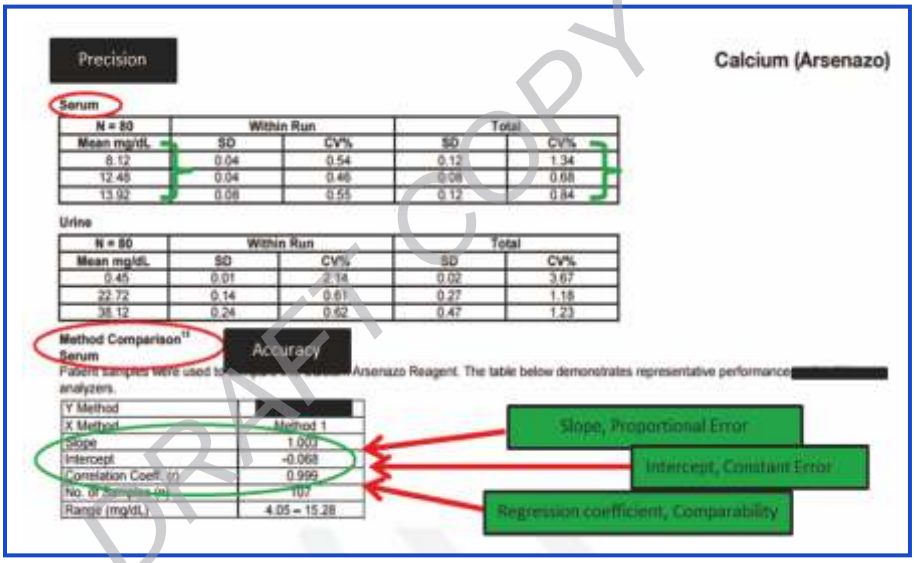


Figure 68: Pre-purchase verification using manufacturer's kit insert (An example)

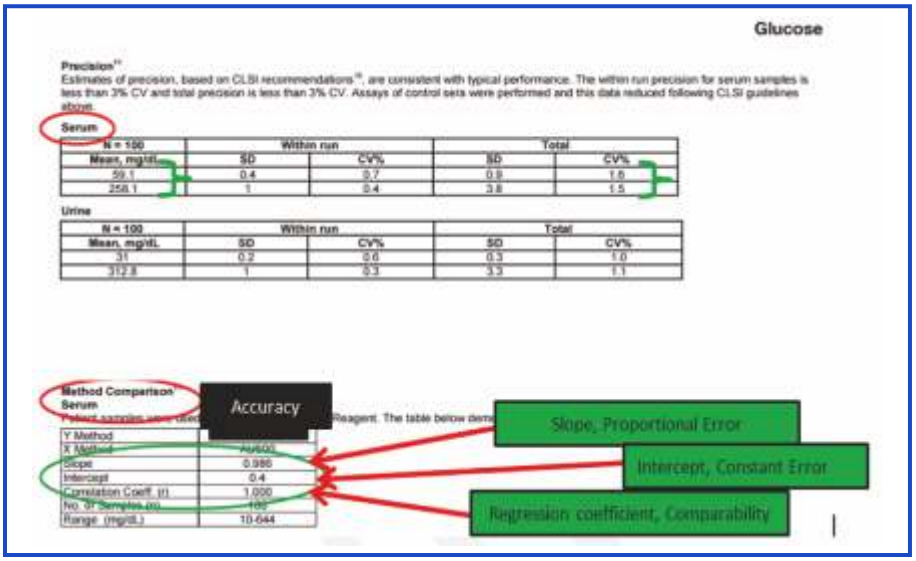


Figure 69: Pre-purchase verification using manufacturer's kit insert (example 2)

The above figures show a product inserts of calcium and glucose for serum and urine. Let us examine the details for serum.

E.g. Calcium

- To arrive at sigma metrics 4 key values are required. Target Value, Observed Value, % CV/ SD and %TE_A/ TE_A
- Precision details: The CV% for Calcium at 3 Clinical Decision Levels, 8.12, 12.48 and 13.2 mg/dL are 1.34. 0.68 and 0.84 respectively. (Green brackets). The Clinical Decision Levels may be considered as the Target Values for which the Sigma is to be assessed.
- Accuracy details are given as method comparison where patient samples were used to compare the method with a standard method. 3 values are to be noted, Correlation coefficient denoting the comparability and the slope and intercept denoting the Proportional and Constant parts of Systematic error. (The details of these are explained along with Method Validation in later sections). Using the formula Y'= mx+b, where m is the proportional error, x is the clinical decision level and b is the constant error, the Y or Y' can be calculated. Y' becomes the Observed/ Obtained value, if the method is used, for the Target Values. From Y', the bias (Systematic Error) and % Bias (%Systematic Error) may be calculated using the formula SE%= (SE/ Clinical Decision Level) *100. % TE_A or TE_A may be chosen from any reliable source. Sigma calculations can be done as shown in figure 71.

	Analyte	Number of papers	Biological Variation		Desirable specification		
			CV_I	CVg	I(%)	B(%)	TE(%)
S-	Calcium	24	2.1	2.5	1.05	0.82	2.55
S-	Calcium, complexed	1	5.3	4.5	2.7	1.7	5.1
U-	Calcium, concentration, 24h	4	27.5	36.6	13.8	11.4	34.1
S-	Calcium, ionized	2	1.7	1.9	0.9	0.6	2.0
S-	Calcium, protein bound	1	4.1	6.1	2.1	1.8	5.2
S-	Calcium, ultrafiltrable	1	2.2	2.7	1.1	0.9	2.7
S-	Carbohydrate deficient transferrin	1	7.1	38.7	3.6	9.8	15.7
P-	CO_2, total	1	4.0	4.8	2.0	1.66	4.96
S-	γ-glutamyltransferase (GGT)	10	13.4	42.15	6.7	11.06	22.11
S-	Globulins, total	1	5.5	12.9	2.8	3.5	8.0
P-	Glucose	1	4.5	5.8	2.3	1.8	5.5
S-	Glucose	15	5.6	7.5	2.8	2.34	6.96
(B)Erythr-	Glucose-6-phosphate-1-dehydrogenase (G6PDH)	1	32.8	31.8	16.4	11.4	38.5
B - spot	Glucose-6-phosphate-1-dehydrogenase (G6PDH)	1	7.3	10.3	3.7	3.2	9.2
P-	Glutamic acid	1	46.4	79.9	23.2	23.1	61.4
P-	Glutamine	1	12.1	22.0	6.1	6.3	16.3
S-	Glutathion peroxidase	1	7.2	21.7	3.6	5.7	11.7

Figure 70: TE_A values from BV for the above examples

Calcium Sigma Calculations									
Clinical Decision Level (X)	Slope (m)	Intercept (b)	Y'=mX+b	SE= Y'-X	Abs	SE% = (SE/X)*100	CV%	TEa % (BV desirable)	Sigma
8.12	1.003	-0.068	8.076	-0.044	0.044	0.537	1.34	2.55	1.420
12.48	1.003	-0.068	12.449	-0.031	0.031	0.245	0.68	2.55	3.705
13.92	1.003	-0.068	13.894	-0.026	0.026	0.189	0.84	2.55	3.004
Glucose Sigma Calculations									
Clinical Decision Level (X)	Slope (m)	Intercept (b)	Y'=mX+b	SE= Y'-X	Abs	SE% = (SE/X)*100	CV%	TEa % (BV desirable)	Sigma
59	0.986	0.4	58.57	-0.426	0.426	0.722	1.6	6.96	4.084
258	0.986	0.4	254.79	-3.212	3.212	1.245	1.5	6.96	2.459

Figure 71: Sigma Calculation for the above examples showing unacceptable Sigma for lower limit of Calcium and upper limits of Glucose

Using the data provided by the manufacturer, and the TE_A as per BV (Desirable), sigma metrics have been calculated for calcium and glucose, at the clinical decision levels, chosen by the manufacturer. The values obtained should be checked against the quality specifications set by the lab. In the example given, calcium method is showing sigma of less than 3 at the lower clinical decision level. Understanding the method's suitability before purchase will enable the lab to decide optimally. Post purchase validation may prove futile in such a situation where the manufacturer's claims itself proves inadequate to meet the requirements of the lab.

4.4 Acceptance Testing/ Method Evaluation/Performance Verification

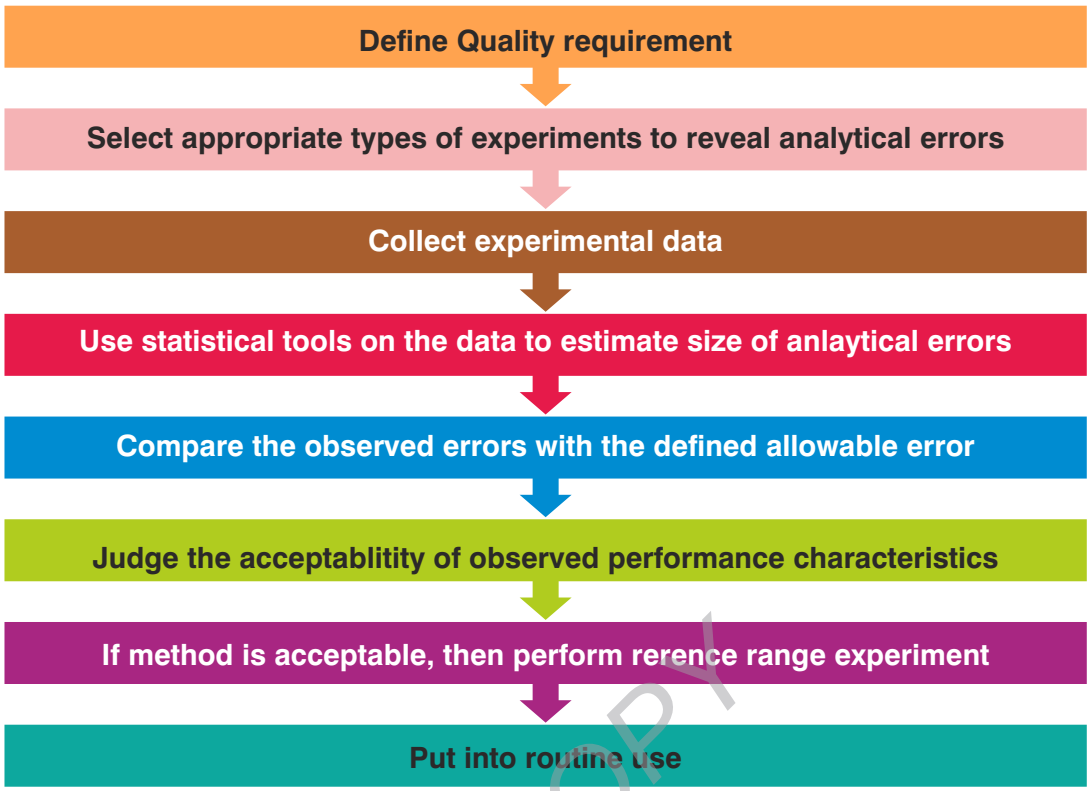
Verification of a quantitative system (for example Chemistry analyzer or Hematology analyzer) consists of an established set of required experiments. Each laboratory should first design a verification plan describing how they will satisfy each of these requirements. The plan must also detail the acceptability criteria for each element.

After completing all of the exercises, results should be compiled and filed in an organized manner.

These records should be retained for the life of the instrument.

A summary should be prepared that contains a place for the Laboratory Director to sign, indicating the validation has been reviewed and approved.

4.5 Verification Plan



4.6 Understanding Quality Requirements

The lab has to define its quality requirements to ensure that the test selected meets intended use for that test. It is the laboratories' responsibility to the define the quality required and then judge the acceptability on the basis of the performance observed in the laboratory against the goals selected. The lab also has to verify the claims by the manufacturer for specific performance characteristics as per quality specified by the lab. (See below as an example, where the laboratory selects the sigma-metric of 3 as the minimum performance quality required for this selected test to meet, before it can be judged acceptable. If the lab chooses the quality requirement as TE = Bias +3 SD, and TE_A is chosen from CLIA, then using the data from the exercises, the lab has to calculate the Total Error using that formula. Bias +3 SD should be less than the CLIA TE_A. Alternatively the lab may choose a defendable and attainable Sigma limit to refer the verification against.

Care must be exercised that the quality specifications chosen should be both attainable and defensible.

"An illustrative example of Quality Specifications of a lab for an analyte."

$TE < TE_A$

Meet or exceed manufacturer's performance specifications and/or

If the lab chooses 3 SD as the quality specification and CLIA as the chosen TE_A, then,

TE= Bias + 3SD < CLIA TE_A

(%TE= %Bias + 3CV% < CLIA $\%TE_A$)

Sigma Metrics; A sigma of >3

or as decided by the quality requirement of the lab for the quality of that analyte

As seen in the ongoing method evaluation, it will be good to evaluate the method for sigma scale, at all clinical decision levels.

4.7 Select Performance Characteristics considered under method evaluation

Ideally seven performance characteristics that should be evaluated before reporting results of a new test method/system as per CLSI guidelines include:

1. Precision
2. Accuracy (measured bias) or comparability (measured differences)
3. Linearity over the measuring interval or analytical measurement range (AMR)
4. Limit of detection (LoD) and limit of quantitation (LoQ or analytical sensitivity)
5. Specificity or interference
6. Reagent or sample (analyte) carryover
7. Reference interval or decision value (interpretive information)

In the following sections, each of these performance characteristics is explained. The definition of the performance characteristic, collecting data for the exercise, running the experiment, data analysis using statistical tools, evaluation of data and acceptability criteria are explained. Finally, drawing the conclusions on method performance by analyzing each data set, verifying it against set quality goals (TE < TEa or meeting the sigma performance), and finally the documentation of the evaluation exercise and introduction to routine service is explained.

4.8 Precision

Precision is the agreement of the measurements of replicate runs of the same sample. Replication experiments are performed to estimate the imprecision or random error of the analytical method. Precision is measured in terms of coefficient of variation (CV).

EP15: a five-day procedure to verify that imprecision meets the claims of a measurement procedure (EP15 is most frequently used by clinical laboratories for method evaluation.)

EP05: a 20-day procedure to establish the imprecision for a measurement procedure.

4.8.1 Things to keep in mind while doing the precision exercise

- Time period: within-run, within-day, day-to-day
- Number of runs of the same sample: minimum of 20
- Sample matrix: patient sample or simulate patient sample
- Analyte concentration: medical decision limit
- Calculations: mean, standard deviation (SD), coefficient of variation (CV)

Precision will be evaluated by running between-day (intermediate precision) using normal and abnormal control samples and within-day (repeatability) precision using patient samples at different clinical decision levels. Between-day precision can be tested by running each QC once per day for 20 days or 4 times a day for 5 days. Within day precision will be tested by running each sample 20 times in one day. The mean, standard deviation (SD), and CV of the replicates will be calculated.

Guidelines for the doing the study: Precision

4.8.2 Short -Term (Within-Run/Day)

A. Sample:

1. Two levels (Low / High or Normal / Abnormal)
2. Patient or quality control

3. Select values near the medical decision point(s) of interest for the analyte

B. Testing:

1. Ensure there is a sufficient reagent to perform all the 20 tests.
2. Run each sample 20 times on the same run, if possible, or least within the same day.

C. Acceptability criteria:

1. Calculate the coefficient of variation (CV) for each level using 20 data points.
2. Compare the calculated CV to the manufacturer's stated precision claims found in the package insert.
3. If manufacturer's precision cannot be met, it is acceptable to attain precision that is <25% of the CLIA Allowable Error or BV Imprecision of Desirable or Minimal.
4. If Short -Term precision is unacceptable, consult the instrument's manufacturer for technical assistance.
5. If unable to resolve issues with short-term precision, the method validation process should be discontinued and a new method selected for potential implementation.

4.8.3 Long-Term (Between-Run/Between Day Labs for Life QC Tool: LJ with CV trends)

A. Material Used:

1. Two / Three levels (Low/High or Normal/Abnormal)
2. Control Material. A lab may already have this data available from their daily QC runs.

B. Testing: Run the QC once a for 20 days or 4 times a day for 5 days to collect minimum 20 data points

C. Acceptability criteria:

1. Calculate the CV for each level using the 20 data points
2. Compare to manufacturer's stated precision claims found in the package insert.
3. If manufacturer's precision cannot be met, it is acceptable to attain precision that is <33% of the CLIA Allowable Error
4. If Long-Term precision is unacceptable, consult the instrument's manufacturer for technical assistance.

4.9 Accuracy [Trueness] (Measured as Bias) ("correlation studies")

Accuracy is the true value of a substance being measured. Verification of accuracy is the process of determining that the test system is producing true, valid results and is expressed numerically as bias.

Estimate of bias or systematic measurement error is done by quantifying the average difference between results from a measurement procedure and results from an accepted reference measurement procedure. When a reference measurement procedure is not available for an analyte, a best-available comparative method may be used to measure bias. Frequently, clinical laboratories perform a comparison of patient sample results between a new and an existing measurement procedure. In the instances where the comparison method is not a reference method, then the trueness of the new method cannot be determined. The laboratory would then be measuring the difference between the methods

and not the bias of the new method. Any difference between the test method and the comparative method must be carefully interpreted.

CLSI Guidelines for Trueness (Measured as Bias) EP15: a method comparison to verify that a new method conforms to a manufacturer's claim for comparability to another procedure. (Minimum of 20 patient samples). EP09: a method comparison to establish a claim for method comparability. (Minimum of 40 patient samples)

4.9.1 Guidelines for the doing the study: Accuracy

a. Determine your comparison or reference method.
   i. The comparison method must be previously validated.
   ii. The comparison method must be currently performing successfully in EQA.
   iii. The ideal comparison method is a similar instrument/method.
   iv. Comparison to an in-house method is preferred if the in-house instrument meets the above criteria.
   v. Samples with known values, such as proficiency testing samples or commercial standards, may be used as the reference method.

b. Sample Criteria
   i. A minimum of 20 samples that cover the reportable range of the method and include points near the Medical Decision Points.
   ii. Patient, quality control, and proficiency testing materials may be used.
   iii. 50% of the selected samples must lie outside of the current reference range.

c. Testing
   i. Run each sample in duplicate on each instrument
      1. Ideally, samples should be run within 2 hours of each other unless the analyte has a shorter stability.
      2. Analyze the replicates (duplicates) in different runs and in a different order.
   ii. Retain the instrument print-outs.
   iii. Duplicates should be averaged.
   iv. Data should be plotted immediately to identify and correct any outliers by reviewing the Comparison Plot or Difference Plot **Labs for Life QC Tool: Accuracy** the Westgard website under Paired Data Calculator.
      - Re-analyze any discrepant results between the test and comparative methods to confirm that the differences are real and not mistakes in recording the values or mix-ups of specimens.
      - If an outlier is identified, then investigate the reason and take corrective action.
      - Document the findings.
      - Remove the outlier from the data set.

d. Time Period of Testing
   i. A minimum of 5 separate days must be used for testing.
   ii. This experiment can be performed simultaneously with the long-term precision study.

e. Evaluation of Data

i. Calculate the slope, Y-intercept, Sy/x, and r. (Explained later)

ii. Evaluate the data.

4.9.2 Checking Correlation and Quantifying Error through Linear Regression

Where accuracy is concerned, 2 major factors should be considered. The degree of agreement or correlation between 2 sets of data and the biases involved despite good correlation. The degree of correlation is expressed as correlation coefficient or **r**. In earlier discussions we have seen that biases in a measurement system are quantified as Systematic Errors (SE). The Systematic errors can be of two types, Constant Error and Proportional Error. In addition, the data includes Random errors (Imprecision)

Linear regression yields all these 4 kinds of data. (In the example below, 3 of these are illustrated). The reference method (red line) and test data (blue line). The yellow table on the left shows the raw data used. (Exaggerated numbers are used to clarify the concept. In real measurements, the differences will be subtle and close observations are required.).

Linear regression consists of finding the best-fitting straight line through the data points of the 2 sets of data.

1. **r.** When 2 sets of data are plotted on a graph with the reference method as the X axis and test method as the Y axis, best-fitting line through these points is called a **regression line**.

   r is a statistical measure of the degree of agreement between 2 sets of data about how close the data are to the fitted regression line. This can be a helpful tool in determining the strength of the relationship between two variables as we can predict scores of one variable from the scores of the second variable. This valuable numerical measure of association between two variables, the **Pearson's or correlation coefficient** or **r**, has a value between -1 and 1 indicating the strength of the association of the observed data for the two variables.

   - 0 says no relationship exists
   - 0 $\geqslant$ 1 explains there is a correlation which is directly proportional
   - 0 $\leqslant$ explains that there is a correlation which is inversely proportional
   - 1 indicates that the model explains all the variability of the response data around its mean.
   - For a method to be comparable, the **r** must be > 0.975

2. **Intercept:** When 2 sets of data are plotted on a graph with the reference method as the X axis and test method as the Y axis, there could be a constant difference between these 2 sets regardless of the concentrations involved. This is called **constant error**. In the example below, each of the test value is 20 points more than reference. Such differences are generally seen as in the case of interfering substances. For the calculation of systematic errors, the formula $Y/Y' = mX+b$ is used where b is the constant error.

3. **Slope:** When 2 sets of data are plotted on a graph with the reference method as the X axis and test method as the Y axis, there could be errors which are proportional to the values. These are called **proportional errors**. In the example below, the test is 40% more than the reference value. For the calculation of systematic errors, the formula $Y/Y' = mX+b$ is used where m is the proportional error

Both constant and proportional errors contribute towards Systematic Errors (Bias)

4. **Sy/x is the random error component** in the calculation of the paired data. This component is not used in method evaluation. The random error from intermediate precision study is used for method evaluation. Sy/x is not further discussed in this module

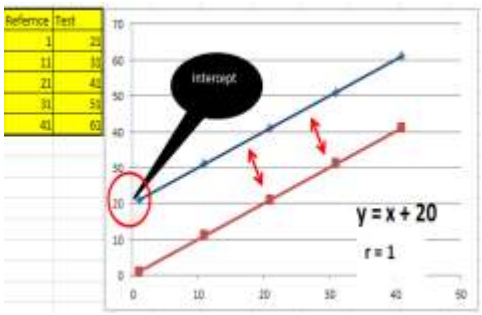
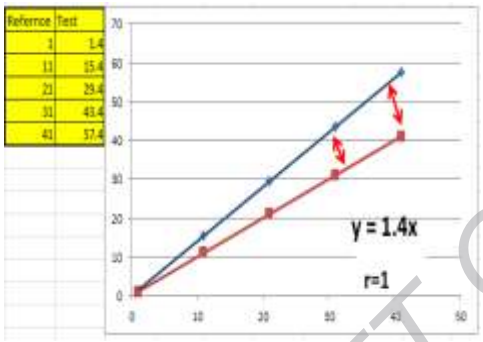
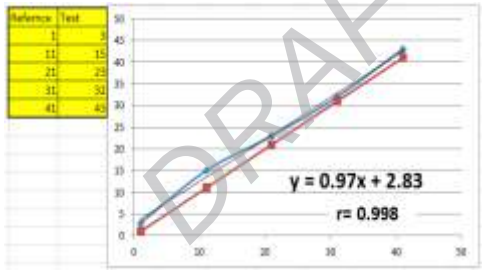
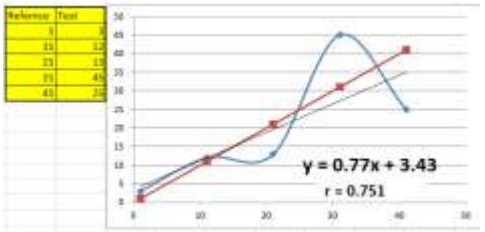
Regression Plots	Interpretations
	**Constant Error: Intercept** There is a constant difference of 20 between the reference and test. The Y' has a constant error of 20. This value is called the intercept or the b in the equation; Y' = mx+b. On the graph, please note the shifting of the blue line away from 0. The constant errors are generally due to interferents. As there is no proportional error, there is no m value in this case. The formula requires addition of b to the X to derive Y'. r = 1 denoting good correlation, regardless of the steep Constant Error. Proceed to Sigma Metrics.
	**Proportional Error: Slope** The test is 40% more than the reference value. The error is proportional all levels and thus the m of the Y' = mx+b. The formula requires multiplication of the value of x by 1.4 to get the predicted Y. The blue and red lines start from 0 but the gap widens as the values increase. There is no b value in this case as there is no constant error r is 1 denoting good correlation despite the proportional error. . Proceed to Sigma Metrics.
	In this graph there is both constant and proportional errors which are quantified by the formula mx+b. These numbers can be used for any predictions of Y. As the r = 0.998, the method is comparable and the predictions are valid. The formula requires multiplication of X by m (0.97) and addition of b (2.83). Proceed to sigma metrics
	Here the r is 0.751. The fact is evident from the visual assessments as these are exaggerated numbers. However, for numbers with smaller differences, the r should be monitored before error calculations are assessed. Any value< 0.975% shows lack of correlation and requires repeat process with more samples.

Figure 72: Explanations for regression plots with illustrative examples

4.9.3 Assessing Acceptability criteria:

Linear regression analysis will be used to determine if the methods are accurate within the specified TEa when the Correlation Coefficient (r) is >0.975. If the Correlation Coefficient/ "r" is < 0.975, then more patient data must be collected. If the Correlation Coefficient remains < 0.975, then paired data calculations or another regression analysis technique needs to be used.

The following process may be adopted for evaluation.

4.9.3 (a) Visual Assessment of Linear Regression graph

Visually inspect the comparison plot for linearity and outliers. Remove outliers. If an outlier is removed, then recalculate the regression statistics. If the regression graph and r are acceptable, proceed as follows.

4.9.3 (b) Determine Bias or Difference between the Methods

Define Medical Decision Points. A Medical Decision Point (MDP) (see below) is the concentration of the analyte at which a medical decision is triggered and/or laboratory established critical values.

1. Using the linear regression equation, calculate the predicted Y/Y' value that corresponds to the concentration of MDP
2. Determine the bias (difference) by subtracting MDP from Y'
3. Calculate the % bias (% difference) as bias/MDP * 100.

4.9.3 (c) Calculate Sigma- metrics.

Using the SD/CV% from the precision experiment, TE_A/ TE_A%, MDPs (See Below) as targets, Bias/ % Bias from the above accuracy experiment, calculate the sigma performance of the new method at each clinical decision point using the formula,

Sigma = (TE_A- Bias)/SD or (TE_A%- Bias %) / CV%. Judge acceptability. as per defined quality specifications of the lab (See below).

The lab should define in its quality specifications about acceptable performance.

In addition to Sigma-Metrics or instead of Sigma Metrics, the lab can opt for comparisons of TE with TE_A as follows

4.9.3 (d) Check TE against TE_A:

TE< TE_A Using the formula TE= Bias+ n* SD or Bias % + n* SD or Bias% + n* CV%. The chosen n is the lab's prerogative. An n of 3 is suggested.

TE= Bias + n* SD < CLIA TE_A

OR

(%TE= %Bias + n* CV% < CLIA %TE_A)

RECAP

TE<TE_A

Meet or exceed manufacturer's performance specifications and/or

TE= Bias + n* SD < CLIA TE_A

(%TE= %Bias + n* CV% < CLIA %TE_A)

And / Or

Sigma Metrics; A sigma of >3

(or as decided by the quality requirement of the lab)

As seen in the ongoing method evaluation, it will be good to evaluate the method for sigma scale, at all clinical decision levels.

Figure 73: Clinical Decision Levels; An excerpt

4.9.3 (e) Medical Decision Point/Clinical Decision Point:

Are those concentrations of the anlayte that makes an impact in clinical decisions. While evaluating methods it is important to check the accuracy at each of these points.
To read more, please check https://www.westgard.com/decision.htm

(Please refer annexure number 3: Medical Decision Points)

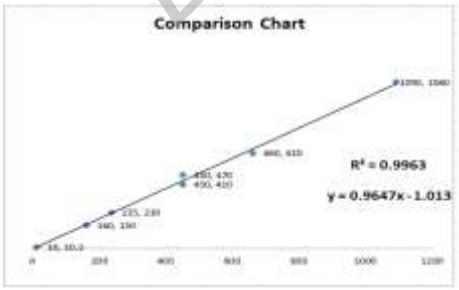
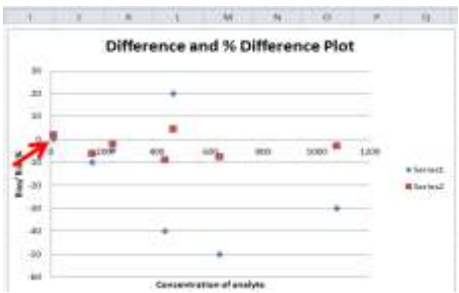
Recap of Evaluation Process

For example, if the method being validated is calcium,

- Computations should be done using 7,11 and 13.5 mg/dL as the target X,
- Using the m and b values from the regression analysis calculate the Y'
- Find the bias/ bias%
- Get the corresponding SD/CV%
- Define the quality requirement (TE_A/ TE_A %)
- Calculate the sigma at each level

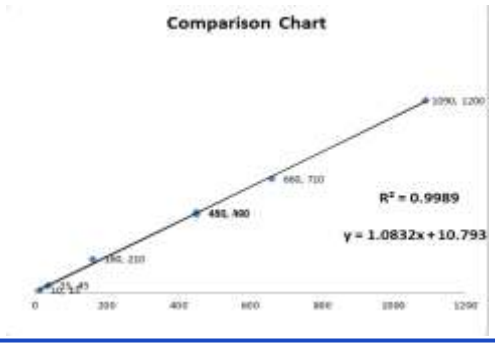
4.9.3 (f) Blandt Altman Plot

In a Blandt Altman plot, in addition to the regression, a difference plot and even a percent difference plot is done. The concept of Blandt Altman is explained below. This can also be easily done using scatter plots on the Excel.

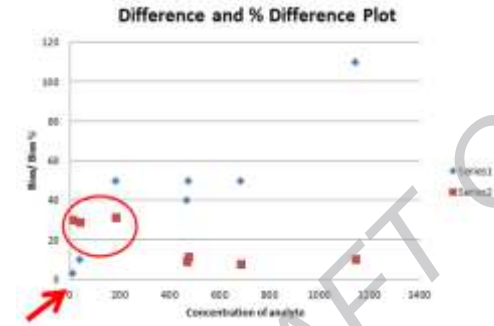
Regression Plots	Interpretations
	A set of 7 data points are given in the chart as an example. A minimum of 20 data sets are required for this exercise. The mean of the 2 values, difference or Bias (Test- Reference), % Difference or Bias (Bias/Reference*100) are calculated. The values range covering the range of performance expected, in this case, from 10-1090
	The data is used to obtain the linear regression. Shows an acceptable correlation of 0.99. Note the m and b values of 0.9647 and -1.013 respectively. The data chart also shows a negative bias (negative b value) for the new method being assessed
	On this plot, both the difference (blue diamonds) and the % Difference (red squares) are plotted. The values are scattered on both sides of 0 (red arrow). However, a negative bias is noticed, with more data points on the negative side. The bias looks exaggerated in the case of difference in units and less pronounced in % difference plot. The difference plots give a quick assessment of the performance visually.

	Reference	Test	Mean	Difference	Difference %
1	450	490	470	40	8.89
2	160	210	185	50	31.25
3	35	45	40	10	28.57
4	660	710	685	50	7.58
5	10	13	11.5	3	30.00
6	450	500	475	50	11.11
7	1090	1200	1145	110	10.09

Another set of data points where the Test values show a considerable positive bias



The R value is still acceptable, but notice the b value has gone up considerably which will affect TE calculations that could push TE > TEa or give a low sigma value



The difference and % difference plots show all values on the upper side of Zero (red arrow). Difference (blue diamonds) and the % Difference (red squares) are all above zero. At the lower concentration of analytes, however the %bias is more pronounced between 20-40%. At higher concentrations, the %bias is less, between 10 and 20%. That the % Bias is more conclusive is evident from the plot. At lower levels, the unit bias is small, but the % Bias is significant. In the case of higher values, this is just the opposite

Figure 74: Illustrative example with explanation for Blandt Altman

Blandt Altman plots thus give bias and % bias plots in addition to the linear regression data and valuable details for visual assessment. The lab is now required to assess the data elicited against its quality specifications. Visually scan for significant and dramatic differences at the upper and lower ends of the range. Positive or negative biases should be addressed by repeating the accuracy exercise. In the event of persistent biases, a reevaluation biological reference range must be done

4.10 Linearity

Linearity studies are performed to determine the linear reportable range for an analyte. The linearity for each analyte is assessed by checking the performance of recovery throughout the manufacturer's stated range of the testing system. This is done using a set of standards containing varying levels of an analyte in high enough and low enough concentrations so as to span the entire range of the test system. Therefore, the demonstration of the linear range requires a series of known concentrations or known relationships established by dilution. A quantitative analytical method is said to be linear when measured results from a series of sample solutions are directly proportional to the concentration or activity in the test

specimens. This means that a straight line can be used to characterize the relationship between measured results and the concentrations or activity levels of an analyte for a determined range of analyte values.

Linearities are performed whenever a new analyzer, analyte, or method is introduced into the laboratory, or when an analyzer is replaced. Linearities may also be performed for troubleshooting purposes when quality control is unacceptable and deviations from acceptable data cannot be explained, when major analyzer repair or replacement of components has taken place, or at intervals prescribed by the manufacturer in the instrument's user manual.

i. **The Analytical Measurement Range (AMR)** is the range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment not part of the usual assay process. AMR validation is the process of confirming that the assay system will correctly recover the concentration or activity of the analyte over the AMR. The manufacturer defines the AMR – but it is the laboratory's responsibility to verify it.

ii. **The Clinical Reportable Range / Reportable Range (CRR)** is the range of analyte values that a method can report as a quantitative result, allowing for specimen dilution, concentration or other pretreatment used to extend the AMR. The laboratory must specify the maximum concentration or dilution that may be performed to obtain a reportable numeric result.

A linearity study is used to establish or verify the measuring interval for a measurement method. Measuring Interval: the interval between lower and upper numerical values for which a method can produce quantitative results suitable for the intended clinical use. The measuring interval is verified by demonstrating a linear relationship between the measured and expected concentration relationships.

CLSI Guideline for Linearity – Measuring Interval EP06: procedures to verify or establish the linear measuring interval of a measurement procedure. An extended procedure is explained here to calculate the acceptability at each level, by calculating the sigma-metrics using the slope and the intercept derived from the linearity plot.

4.10.1 Sample Criteria

1) A minimum of 5 samples that cover the reportable range of the method.
2) When plotted, the values should ideally be equidistant from each other.
3) Quality control, commercial linearity standards, and calibrators (if a different lot number is used to calibrate the instrument) may be used.
4) Patient specimens may be used if a high value near the expected upper range can be found.
5) Sufficient volume of each sample must be available to analyze in triplicate and for possible troubleshooting.

4.10.2 Sample Preparation

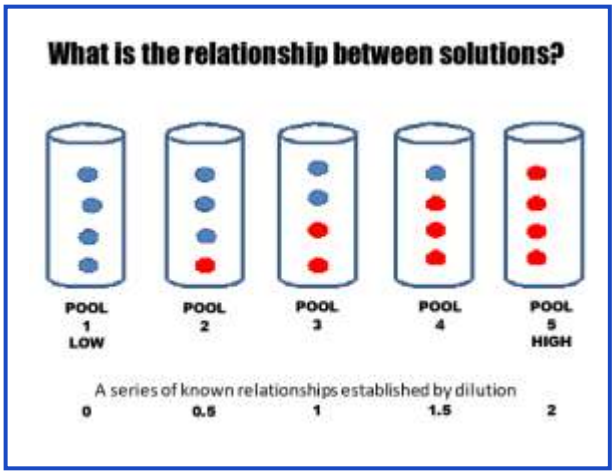


Figure 75: Making serial dilutions for linearity test

1) If using purchased materials, refer to manufacturer's instructions.
2) If using patient specimens, then perform the dilutions using the manufacturer's recommendation of the diluent to use with out-of-range specimens.
3) Select a patient specimen near the detection limit and another patient specimen near or slightly above the expected upper limit of the working range. Ensure that both specimens meet storage and stability requirements as stated by the manufacturer.
4) Prepare 5 pools for testing as follows:
   i. Label the low specimen Pool 1 and the high specimen Pool 5.
   ii. Prepare Pool 2 (75/25) with 3 parts Pool 1 + 1 part Pool 5.
   iii. Prepare Pool 3 (50/50) with 2 parts Pool 1 + 2 parts Pool 5.
   iv. Prepare Pool 4 (25/75) with 1 part Pool 1 + 3 parts Pool 5.
   v. Pool 5 is the High sample
6) Care must be taken to mix each pool thoroughly, and to protect the pools from evaporation or other deterioration.

4.10.3 Running Samples, Plotting Graphs

1) Samples will be run in triplicate.
2) The mean value for each point will be calculated.
3) If one value deviates greatly from the others due to random error, it may be removed from the data analysis and repeated.
4) Data should be plotted immediately to identify and correct any outliers.
5) Save the instrument print-outs to be filed with the summary statistics

4.10.4 Evaluation of data:

1) Determine the Assigned Value (X) for each data point:
   If standards have known values, then insert them into the Assigned Value (X) column following the manufacturer's instructions.

   If using patient dilutions,

   a) Pool 3 will be used as a true value; therefore, the mean value (Y) obtained will be the assigned value (X). (Red horizontal arrows in yellow highlighted columns in Fig 76 depicting how the Pool 3 mean will be the anchor value from where other targets are assigned)

   b) The remaining pools will be calculated using the known relationship between dilutions as follows:

I. Pool 1 = mean of Pool 3 x 0 = 0 (Pool 1 must be zero or near zero, or else the actual value must be taken into account)
II. Pool 2 = mean of Pool 3 x 0.5
III. Pool 4 = mean of Pool 3 x 1.5
IV. Pool 5 = mean of Pool 3 x 2.0

2) The values obtained are then fed and the mean calculated. (**Blue downward arrows**)

3) The recovered mean values will be plotted versus the corresponding assigned values. A best-fit straight line will be drawn to connect the points on the graph with greater emphasis on the first three points when drawing the best-fit line. Alternatively, the scatter plot may be used on excel or **Labs for Life QC Tool: Linearity** may be used. Yet another alternative to creating a graph is to use the Linear-data Plotter located on the **www.westgard.com website.**

4) The plot will be visually inspected for a linear relationship. If using a paper plot, you may not be able to go further. The visual inspection for linearity would also suffice.

5) If using a scatter plot on Excel/ Labs for Life/ Westgard, note the Slope and Intercept derived from the regression graph.

6) Y' is calculated using formula Y' = mX + b. (Green Highlighted Column)

7) Ideally, the slope is equal to 1.0. Acceptable Range Guideline: 0.9-1.1

8) If the slope is outside the acceptable range, examine the results of the highest standard first. It is possible that the test is nonlinear at its highest value

9) Ideally, the Y-intercept is equal to zero.

10) For enzyme determinations and other assays with results in high numerical values, the Y—intercept may be much higher with no clinical significance. (In the figure below, the intercept is 5.6) The Y— intercept for assays with low numerical values should be 0.0 +/— 1.0

11) The predicted Y (Y') value will be subtracted from the associated recovered/observed mean value (Y-Y'). (Lavender Highlights, column 1) to get the absolute difference

12) % Difference will be calculated by the formula, (% Difference = (+/- Diff / y')* 100/ Predicted Mean)* 100. This difference is the systematic error due to non-linearity. (Blue Highlight)

13) Systematic error will be compared to 50% of the total allowable error (TE_A:Yellow Highlight)

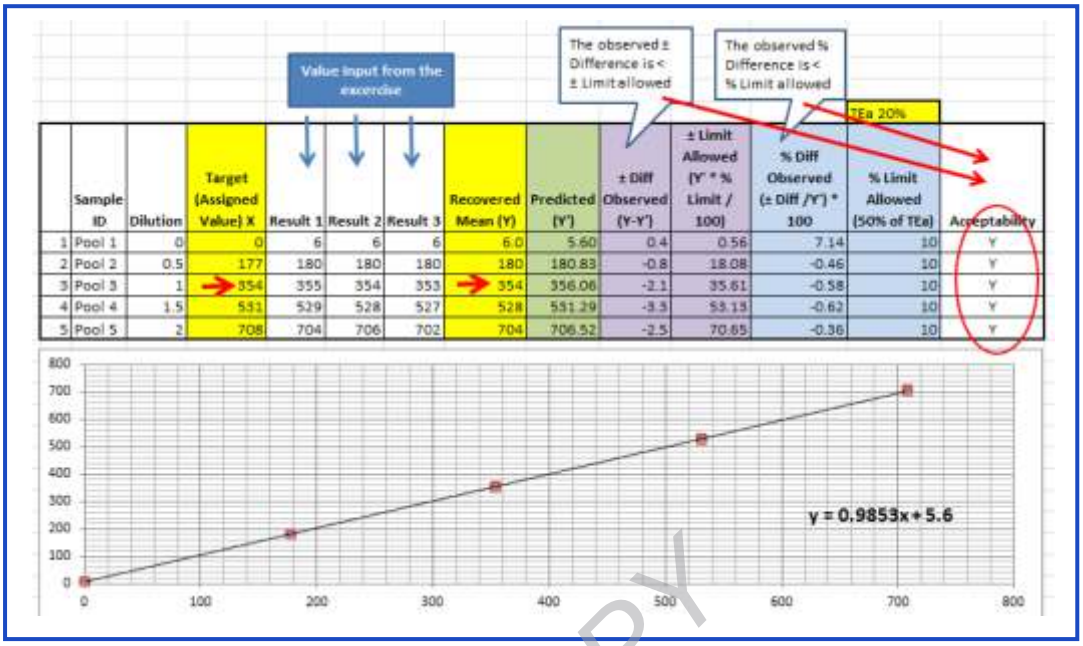
4.10.5 Acceptability criteria:

1) Visual assessment of the best-fit line on the linearity plot must demonstrate a linear relationship. Calculate % Limit and the result should confine to 50% of the selected quality requirement. Example as, by dividing the CLIA % TEa by a factor of 2.

2) Calculate ± Limit by either inserting 50% of the CLIA absolute value or by multiplying the %Limit by Y', whichever is greater.

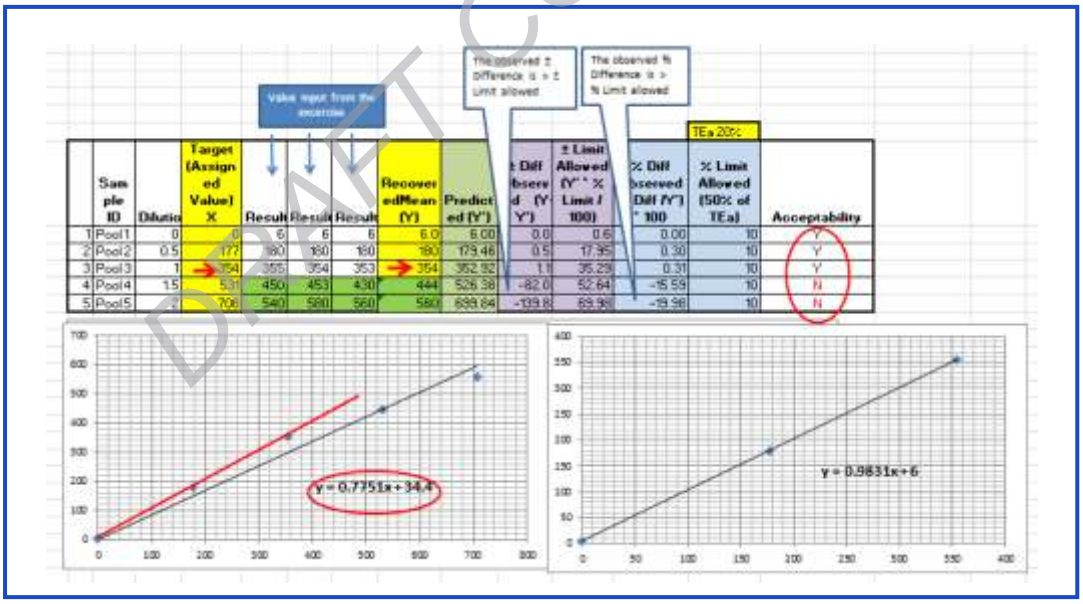
3) Compare that systematic error to 50% of the total allowable error. The systematic error must be less than 50% of the total allowable error.

In the example below, a method is evaluated for linearity. Assume that the manufacturer's claim is 5-700. The lowest recovered value however is 6. The mean of the highest is 704. The method is linear and acceptable at all clinical decision levels.



	Sample ID	Dilution	Target (Assigned Value) X	Result 1	Result 2	Result 3	Recovered Mean (Y)	Predicted (Y')	± Diff Observed (Y-Y')	± Limit Allowed (Y' * % Limit / 100)	% Diff Observed (± Diff /Y') * 100	% Limit Allowed (50% of TEa)	Acceptability
1	Pool 1	0	0	6	6	6	6.0	5.60	0.4	0.56	7.14	10	Y
2	Pool 2	0.5	177	180	180	180	180	180.83	-0.8	18.08	-0.46	10	Y
3	Pool 3	1	354	355	354	353	354	356.06	-2.1	35.61	-0.58	10	Y
4	Pool 4	1.5	531	529	528	527	528	531.29	-3.3	53.13	-0.62	10	Y
5	Pool 5	2	708	704	706	702	704	706.52	-2.5	70.65	-0.36	10	Y

Figure 76: Illustrative example of a linearity test. The test is linear and the error within limits at all dilutions



	Sample ID	Dilution	Target (Assigned Value) X	Result	Result	Result	RecoveredMean (Y)	Predicted (Y')	± Diff Observed (Y-Y')	± Limit Allowed (Y' * % Limit / 100)	% Diff Observed Diff /Y') * 100	% Limit Allowed (50% of TEa)	Acceptability
1	Pool 1	0	0	6	6	6	6.0	6.00	0.0	0.6	0.00	10	Y
2	Pool 2	0.5	177	180	180	180	180	179.46	0.5	17.95	0.30	10	Y
3	Pool 3	1	354	355	354	353	354	352.92	1.1	35.29	0.31	10	Y
4	Pool 4	1.5	531	450	453	430	444	526.38	-82.0	52.64	-15.59	10	N
5	Pool 5	2	708	540	580	560	560	699.84	-139.8	69.98	-19.98	10	N

Figure 77: Illustrative example of a linearity test. The test is linear in the first three dilutions.
The error within limits in the first three dilutions only. The limits of linearity, in this case is less than the manufacturer's claim.

In figure 77, the dilutions yield non-linear values at higher levels. As mentioned earlier, if the slope is outside the acceptable range, examine the results of the highest standard first. It is possible that the test is nonlinear at its highest value. In the above example, Pool 4 and 5 values are out of linearity. A regression graph shows unacceptable slope and intercept values. However, a line joining the lower points and the regression plot of the same acceptable values. Fig 77, graph on the right after removing the higher values. In this case the validated linearity is up to 354. The higher values exceed acceptable limits in comparing with the 50% of TE_A.

4.10.6 Linearity, Analytical Measurement Range and Clinical Reportable Range

In the above example, (fig 76) the manufacturer's claim is 5-700. The lowest recovered value however is 6. The mean of the highest is 704. However, the AMR is only 6-700 as the upper end of AMR cannot be more than the Manufacturer's claim. However, the lab can report an analyte beyond the AMR by diluting sample. This range is called the Clinical Reportable Range/ Reportable range. CRR depends upon the lab's decision to allow dilutions. The dilution factors must be clearly mentioned in the SOP. For example, if a 1: 9 dilution is performed, then the CRR in the above example is 700*10= 7000. Below and beyond this the lab will report as <6 or >7000.

Note the following terminology and corresponding figures as per the example above:

1. Manufacturer's Claimed AMR: 0-700
2. Linearity Range: 6-704
3. Validated AMR: 6-700
4. Clinical Reportable Range: 6-7000

4.11 LoD / LoQ Limit of Detection (LoD) & Limit of Quantification (LoQ) (sometimes referred to as "Analytical Sensitivity")

LoD/ Sensitivity: the lowest amount of analyte (measurand) in a sample that can be detected with a stated probability. Sensitivity is the lowest concentration of an analyte that can be measured. For an FDA approved, unmodified method, the manufacturer's stated sensitivity may be used. However, the LoD will be verified for immunoassays, therapeutic drugs, drugs of abuse, cardiac markers, and tumor markers. LoQ: the lowest amount of analyte (measurand) in a sample that can be quantified with acceptable precision and bias under stated experimental conditions.

Usually, laboratories review and accept the manufacturer's claims for LoD and LoQ. But these characteristics can be tested by laboratories using: CLSI Guideline for LoD and LoQ EP17: procedures for verifying or establishing the LoD and the LoQ. This module is not explaining this concept further.

4.12 Interference / Specificity

It is an artefactual increase or decrease in the apparent quantity of an analyte due to the presence of a substance that reacts nonspecifically with the measuring system. It is the determination of the effect of interfering substances. Most manufacturers evaluate a large number of substances known or suspected to be potential interferents. They report this information in the Instructions For Use (IFU). It is not practical for most clinical laboratories to repeat such an investigation and inspection of the manufacturer's information is frequently sufficient. For an FDA approved, unmodified method, the manufacturer's stated specificity can be used.

But these characteristics can be tested by laboratories using: CLSI Guideline for Interference EP7: procedures for testing constant error due to interference. This module is not explaining

this concept further.

4.13 Carryover

The discrete amount of reagent or analyte carried by the measuring system from one test into subsequent test(s), thereby erroneously affecting test results.

Periodic carryover assessment is warranted in CBC analyzers. Reagent carryover among different measurement procedures on multichannel automated biochemistry analyzers is an evaluation that is usually conducted by measuring system manufacturers. This characteristic can be tested by laboratories using CLSI Guideline for Carryover (EP10: includes an assessment of sample carryover along with other parameters). Some more details of this is explained in 4.15, page 93. **Labs for Life QC Tool: Carryover**

4.14 Reference Intervals

Interpretive information for laboratory test results that is frequently provided as the central 95% interval of results for a group of well-defined reference individuals. Thus BRI (Biological Reference Interval) is the range of test values expected for a designated population where 95% of the individuals are presumed to be healthy (or normal).

When a new analytical equipment is installed the Biological Reference Range relevant for the target population should be determined. Laboratories can produce reference intervals in a variety of ways, including testing procedures found in CLSI Guideline for Reference Intervals or Decision Value C28.

Procedures for establishing a reference interval are

- Verifying the suitability of a manufacturer-proposed reference interval
- Transference from the previously used reference interval by using the slope and intercept from the accuracy testing
- Establishing a new reference interval

As said before, the Reference Interval (or Reference Range) is the range of test values expected for a designated population in which 95% of the individuals are presumed to be healthy (or normal). In some analytes reference interval have been replaced by decision limits established by international consensus. For example, cholesterol (NCEP) and HbA1c (ADA). For such analytes there is no need establish or verify the reference intervals. For such analytes, there is no need to establish de novo or even verify the reference intervals. Rather, laboratories must concern themselves with the accuracy of the results they report; that is, that cholesterol values they report are not appreciably different from the values that are reported by a certified reference laboratory on the same samples. For such analytes, the onus falls on manufacturers to ensure their methods are traceable and on individual laboratories to ensure they run those methods correctly (using peer group, quality control, proficiency testing, etc.)

In instances, if medical decision limits will be used for interpretation; ensure the method being used has validated reference intervals traceable to certified reference material and the accuracy of your method at those medical decision levels is maintained. You should cite the source of the medical decision limits to be used by your organization, in your reports.

Even though the results may be accurate and precise, reported results may be clinically misleading if the BRI is not fit for the population served.

4.14.1 Verification of Reference Interval

The primary process while considering reference interval is verification.

When verifying a reference interval, ensure the comparability of the test subject population. If there are substantial differences in the geographic locations or demographic variables of the two populations that are known to cause differences in the reference values, then a reference interval must be established.

Select reference range to be verified. This may include Current laboratory ranges, Manufacturer's ranges, Published reference ranges or locally established reference ranges

Determine population to be used to verify reference range.Qualify healthy volunteers. This is the most important step and can be done through a questionnaire or health assessment. Obtain samples from 20 healthy participants for each range to be verified. Test each sample immediately and evaluate.

If	Then
90% of samples are within the reference range	The reference range is verified.
< 90% of samples are within the reference range	Re-evaluate the range being verified. Re-evaluate the healthy volunteer qualifications. Collect and evaluate 20 additional samples.
90% of the additional samples are within the reference range	The reference range is verified.
< 90% of the additional samples are within the reference range	Proceed Establishment of Reference Ranges or Transference of reference ranges

4.14.2 Establishment of Reference Ranges

As in the verification step, select healthy volunteers through questionnaires.

Obtain samples from 120 healthy participants for each range to be verified. The 40 samples previously collected in step I above can be used as part of the 120 samples. Test each sample immediately after collection and evaluate. It is not advisable to collect and test all samples on the same day.

Evaluation of data

Plot the data in a histogram and visually evaluate the frequency distribution and outliers.

Eliminate outliers based on visual examination and clinical experience.

Use a non-parametric method to determine the reference range.

Rank (order by size) the values from lowest to highest. Example:

Female Calcium Results (mg/dL) *(Data from samples 6 - 115 omitted for example purposes)*			
Sample 1	8.8	Sample 116	10.1
Sample 2	8.9	Sample 117	10.1
Sample 3	**8.9**	**Sample 118**	**10.2**
Sample 4	8.10	Sample 119	10.3
Sample 5	8.11	Sample 120	10.4

Multiply the total number of samples +1 by 0.025 to determine the sample number that represents the low end of the range.

Example: Total number of samples= 120.
Low end = (120 + 1) x 0.025 = 3.025 = 3.
Sample 3 is the low end: **8.9 mg/dL.**

Multiply the total number of samples +1 by 0.975 to determine the sample number that represents the high end of the range.

Example: Total number of samples= 120.
High end = (120 + 1) x 0.975 = 117.975 = 118.
Sample 118 is the high end: **10.2 mg/dL**

Use these rank values to estimate the upper and lower reference limits.
Example: Reference range is "Sample 3 to Sample 118" or **8.9 - 10.2 mg/dL**

Since the assumption is that 95% of the population is healthy, removing 2.5% from the upper and lower ends enables you to include the 95% group.

4.14.3 Transference of Reference Ranges without Verification

Labs for Life QC Tool: Reference Range by Transference

The CLSI C28-A2 describes different ways for a laboratory to validate the "transference" of established reference intervals. Pediatric reference intervals often require this approach because of the difficulty in obtaining sufficient specimens to establish or verify reference intervals. If a laboratory wishes to transfer a reference interval established by another laboratory or publication, the acceptability should be assessed based on several factors: similarity of geographies and demographics, similarity of test methodology, sound clinical judgment and consultation with local medical professionals. Approval by the laboratory medical director is required and must be documented. Using the slope and intercept obtained from the accuracy experiment, and the Lower and Upper Reference range from the previously validated method, using the Y= mx+b equation, the new upper and lower ranges may be derived.

If slope is 0.97 and Intercept is 2.83, the current reference range is 12-50, then the new range is;	
12	=(12*0.97)+2.83 = 14.5
50	=(50*0.97)+2.83 = 51.0

The BRI for the new method will be 14.5 to 51.0 by transference method. However, it is not advised to do it more than once that is, for one change with reference to one previous method.

4.14.4 Prothrombin Time; Defining the mean and reference range **Labs for Life QC Tool: Coagulation**

2 numbers are very important in the standardization of PT results. ISI and INR. This is because the thromboplastin reagent used for PT estimation is very variable in its strength. PT is a test that needs continuity. A patient on anticoagulants will need repeated estimations of PT. So the variability within the same lab and between labs has to be minimized.

An ideal Thromboplastin will be the same as the standard PT reagent established by WHO. Since in real practice this is not possible 2 corrective steps are undertaken.

1. Each Thromboplastin is required to be calibrated against standard PT reagent established by the WHO and this value is called the ISI or International Sensitivity Index. ISI value has to be assigned by the manufacturer for each lot of reagent. The lower the ISI the more sensitive the reagent. ISI of 1.8 to 2.4 = Low sensitivity, ISI of 1.4 to 1.8 = Average sensitivity, ISI 1.0 to 1.4 = High Sensitivity. Always look and understand the ISI value whenever you get a new lot of PT reagent.

2. INR or International Normalized Ratio: Every lot of thromboplastin is also required to have a population mean from the normal population. For this an estimation of MNPT or Mean Normal Prothrombin Time is required. A laboratory can estimate the MNPT from a minimum of 20 healthy individuals with a relatively equal mix of both sexes over a range of age groups. (Avoid people on anticoagulants, pregnant women, and people with known bleeding tendencies). Estimation of a geometric mean is to be preferred to the arithmetic mean. MNPT samples must be fresh. The mean of a laboratory normal control is not an acceptable substitute for the MNPT, since control samples may differ excessively from each other, particularly in the case of less responsive reagents. The MNPT should be determined with each new lot of PT reagent.

Once the MNPT is known, INR can be calculated by this formula

INR= (Patients Value/ MNPT Value) ^ ISI

Using MNPT data to define Biological Reference Range. The reference interval is calculated by determining the 95% Confidence interval of a group of normal donors. Ideally a number closer to 120 is required. However, the same group that was used for MNPT will serve as the pool for determination of reference interval.

a) Look at the individual PT result
b) Calculate mean and +/- 2 SD range
c) Exclude all those outside 2 SD
d) Now recalculate Mean +/-2 SD
e) This is the ref interval.
f) This reference interval is used in the reports
g) For good thromboplastins the reference interval falls between 10-13 secs

4.15 Carryover: **Labs for Life QC Tool: Carryover**

Carryover is the effect of a previous sample on the next sample. Carryovers interfere with the results. It is very important to estimate the amount of carryover. Of any test. This is particularly

important in CBC Analyzers.

Carryover % estimation in a CBC analyzer

Run any sample with high values 3 times consecutively followed by any sample with low values 3 times consecutively and using the formula given below calculate the carryover%.

[(L1-L3) / (H1-H3)] *100

Alternatively any sample 2 times followed by 3 runs of cell pack and apply the following formula

[(Blank 1- Blank 3) /Sample 2]*100

Maximum allowable carryover % is WBC <2%, RBC < 1%, HB <2%, and Platelets<2%

4.16 Documentation of Method Evaluation

At the end of method evaluation, the lab must have all the activities well documented. These should include the raw data, evidence of all statistical calculations and most importantly, the validation summary report with approval of the lab director authorizing the introduction into routine service. A sample method evaluation summary is given as annexure

It is advisable to start with linearity, then precision, accuracy and finally reference range verification/establishment/transference. The sensitivity (LOD) and specificity (Interferences) specified by the manufacturer maybe used. The carryover exercise maybe carried out periodically, say once in 6 months.

Please refer the annexure number 7: Evaluation Summary Report

PART 3

CONTINUAL IMPROVEMENT

ISO 15189: 2012 (Clauses 4.9 to 4.12)

CHAPTER 5: GENERAL CONCEPTS IN QUALITY ASSURANCE

Learning Objectives

At the end of this chapter the learners will be able to understand the

- The concepts in process control going beyond the testing areas
- Some management tools that can be used within the labs to increase efficiency, detect errors and minimize risks.

5.1 Introduction

There are many process control techniques that come as handy tools to increase the efficiency of a lab and reduce the risk to results, staff and environment. Every technique or tool is unique and has its strength to give output. The most critical point is the selection of techniques best suited for that particular objective as not all techniques can be used everywhere. There are overlapping among the tools and the lab may decide on using which tool and where.

This chapter will help the readers in getting ideas about the following process control techniques

a. PDCA
b. 5s
c. Trend Analysis
d. Root Cause Analysis
e. FMEA
f. Pareto Analysis
g. Value Stream Mapping

5.2 PDCA (Plan, DO, Check, Act)

PDCA is a continuous improvement tool and also called Deming Cycle and Shewart Cycle.

Walter Shewhart

Discussed the concept of the continuous improvement cycle (Plan Do Check Act) in his 1939 book, "Statistical Method from the Viewpoint of Quality Control.

W. Edwards Deming

Modified and popularized the Shewart cycle (PDCA) to what is now referred to as the Deming Cycle (Plan, Do, Study, Act).

It is **an iterative methodology** for implementing improvements, and it has four components

Plan (establish plan and expected results, what? how?)
Do (implement plan, to get the expected output)
Check (verify expected results achieved, analyze the output)
Act (review and assess; or do it again, implement the analysis)

PDCA Process

Component	How to use		Approach
	What?	How?	
P	• Identify the problem to be examined • Formulate a specific problem statement to clearly define the problem • Set measurable and attainable goals • Identify stakeholders and develop necessary communication channels to communicate and gain approval	• Brainstorm potential causes for the problem • Divide overall system into individual processes -map the process • Collect and analyze data to validate the root cause • Formulate a hypothesis • Verify or revise the original problem statement	• Direct observation of process • Process mapping • Flowcharting • Cause and Effect diagrams • Pareto analysis
D	Develop Solutions	• Establish experimental success criteria • Design experiment to test hypothesis • Gain stakeholder approval and support for the chosen solution	• On job training • Stakeholder management & communication
	Implement a Solution	• Implement the experiment/solution on a trial or pilot basis	
C	Evaluate The Results	• Gather/analyze data on the solution	• Direct observation of process • Graphical analysis • Control • Key performance indicators
	Achieve the desired Goal	• If YES go to act • Else go to plan, revise action/problem statement	
A	Implement the full scale solution	• Identify systemic changes and training needs for full • Plan ongoing monitoring of the solution • Continuous improvement • Look other improvement opportunities	• Process mapping (new process) • Standardization of work and process • Visual management • Error proofing • Formal training

Areas to Use these PDCA in a lab

Quality Management Systems: All lab process require PDCA cycle.

- **Plan:** Use a lab standard, for instance ISO 15189/CLSI, to do the planning and establishment of a lab QMS
- **Do:** After establishing the QMS, implement it through SOPs, trainings and capture results as records
- **Check** the recorded results in a scheduled manner. Do internal audits using a checklist (NABL 217 / LQMS / SLMTA)
- **Act:** As per the output during the checking, modify and amend processes

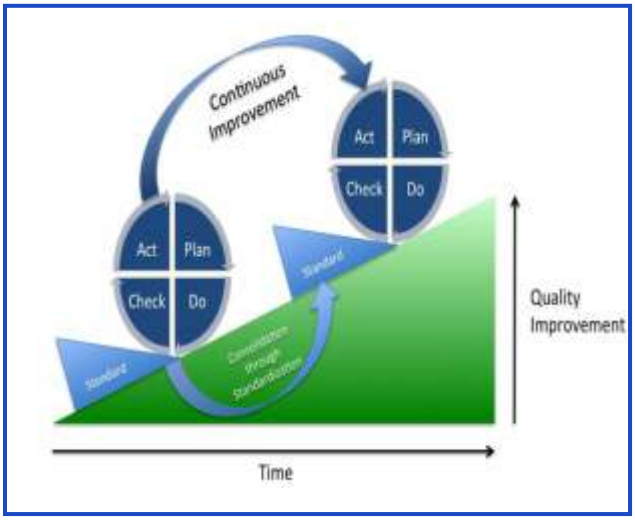


Figure 78: PDCA cycle for Continual Improvement

Process to remember

- The PDCA cycle can be an effective and rapid method for implementing continuous improvement.
- Each step: Plan, Do, Check, and Act are critical for consistent implementation of successful process improvements.
- Avoid the common disconnects as commonly observed, such as over/under-planning and not validating the hypothesis, even on successful results.
- Different organizations will use the cycle uniquely, but organizations that use it well develop tools around PDCA to use it effectively

5.3 The 5S

5S was developed in Japan and was identified as one of the techniques that enabled Just in **Time (JIT) manufacturing,** aimed at reducing turnaround time.

The goal of 5S is to create a work environment that is clean and well-organized. It consists of five elements:

Sort (eliminate anything that is not truly needed in the work area)

Set in Order (organize the remaining items)

Shine (clean and inspect the work area)

Standardize (create standards for performing the above three activities)

Sustain (ensure the standards are regularly applied)

It should be reasonably intuitive how 5S creates a foundation for well-running equipment. For example, in a clean and well-organized work environment, tools and parts are much easier to find, and it is much easier to spot emerging issues such as fluid leaks, material spills, metal shavings from unexpected wear, hairline cracks in mechanisms, etc.

Elements of 5S

Sort

- Remove unnecessary items and dispose of them properly.
- Reduce chances of being disturbed with unnecessary items.
- Prevent accumulation of unnecessary items.
- Remove all parts or tools that are not in use.
- Need fully skilled supervisor for checking on regular basis.
- Don't put unnecessary items at the workplace & define a red-tagged area to keep those unnecessary items.

Set

- Arrange all necessary items so that they can be easily selected for use
- Ensure first-come-first-served basis
- Make workflow smooth and easy

Shine

- Clean your workplace completely
- Prevent machinery and equipment deterioration
- Keep workplace safe and easy to work
- Keep workplace clean and pleasing to work in
- Must be able to detect problems in 5 seconds within 50 feet.

Standardize

- Standardize the best practices in the work area.
- Maintain everything in order and according to its standard.
- Everything in its right place.
- Every process has a standard.

Figure79: Diagrammatic representation of 5S

Sustain

- To keep in proper working order
- Also translates as "do without being told"
- Perform regular audits
- Training and Discipline
- Training is goal oriented process. Its resulting feedback is necessary monthly

New paradigm has included one more S which is "Safety".

Please refer to the Labs for Life Facility Management and Safety module for more on this.

Areas where 5S can apply in a Lab

- Lab Safety
- Lab Quality
- Equipment Management
- Documentation
- Others

5.4 Failure Modes and Effects Analysis (FMEA) Tool

Failure Modes and Effects Analysis (FMEA) is a systematic, proactive method for evaluating a process to identify where and how it might fail and to assess the relative impact of different failures, in order to identify the parts of the process that are most in need of change. FMEA includes review of the following:

Steps in the process

- Failure modes (What could go wrong?)
- Failure causes (Why would the failure happen?)
- Failure effects (What would be the consequences of each failure?)

Teams use FMEA to evaluate processes for possible failures and to prevent them by correcting the processes proactively rather than reacting to adverse events after failures have occurred. This emphasis on prevention may reduce risk of harm to samples, patients and staff. FMEA is particularly useful in evaluating a new process prior to implementation and in assessing the impact of a proposed change to an existing process.

Failure Modes and Effects Analysis (FMEA) was developed outside of health care and is now being used in health care to assess risk of failure and harm in processes and to identify the most important areas for process improvements.

An example of using FMEA in the lab:

Identify a process that needs improvement

Identify the components and classify it on a grid (in rows)

Include the following in columns: Occurrence (Occ), Severity (Sev), Detection (Det), and Risk Priority Number (RPN). Add responsibility, action take, and approximate date for closure also into the columns

In the following example of pre-analytical process in being analyzed

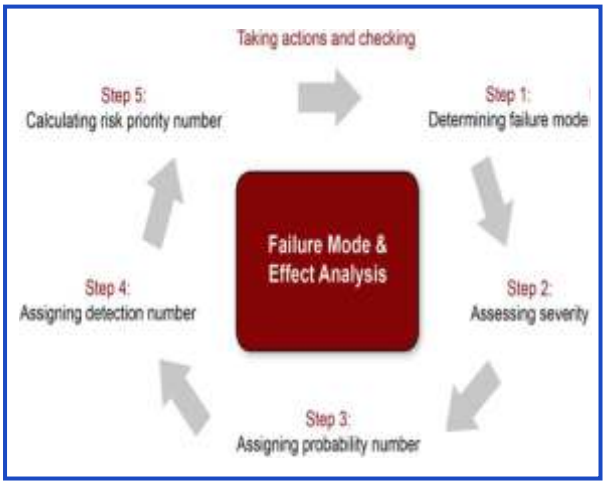


Figure 80: Diagrammatic representation of FMEA

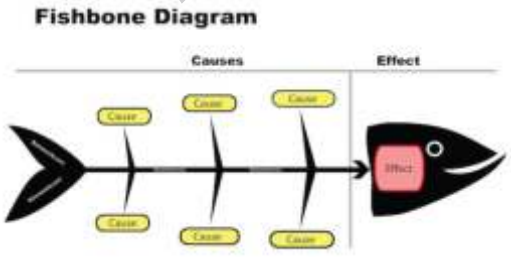
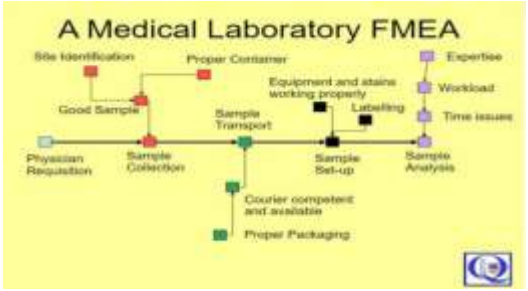
Following the thoughts above, it is clear that a needle stick injury or spillage is self-evident, easily detected, reported and gets a higher score for Det. However, the severity (Sev) is

higher for needle stick as the hazards are more. A hemolysis is less easily reported if the staff is not trained, and includes the risk of erroneous reports. Micro clots which results in probe block, wrong results is even less easily reported. These two can be high in occurrence if the collection practice is compromised. After plotting the grades on the grid, multiply the 3 captured numbers so as to derive the RPN or Risk Priority Number to decide on the priority of interventions. Decide on the course of action and assign responsibility. Track all the risk factors as Quality Indicators.

Process Mode	Occurrence 1-10	Detection 1-10	Severity	RPN = (Occ*Det* Sev)	Action	Responsibility
Needle stick	3	9	10	270	Training, PEP	HOD, QM
Spillage	2	8	8	128	Training, Engineering Controls	HOD, QM
Hemolysis	7	3	8	168	Training, Adequate Phlebotomy equipment	QM
Wrong Container	2	7	8	132	Training	QM
Inadequate Volume	1	3	6	18	Training, Volume Checks	QM
Micro clots	8	5	8	320	Equipment Maintenance, Training	QM
XYZ	2	1	3	6	ABC	Senior Technician

The Detection (Det.) is deleted in many analysis (see below) as it may bring down the RPN spuriously. Only Occurrence and Severity are considered. Not being able to detect the risk factors is not to the advantage.

FMEA can be used simultaneously with a Fishbone matrix and Risk Prioritization matrix in understanding and eliminating risks

	A fishbone diagram is typically used in root cause analysis. It can also be used for risk analysis.
	A fishbone chart of all pre-analytical process is made. 4 key points, requisition, sample collection, transportation, sample set up (accessioning) and analysis have been mapped. Any process can thus be mapped.

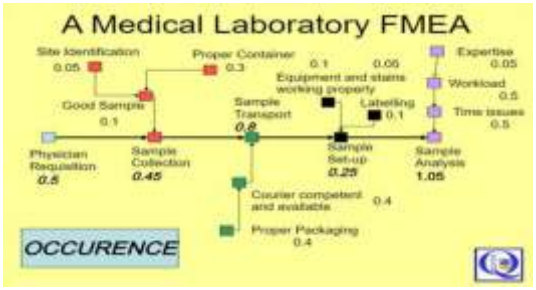
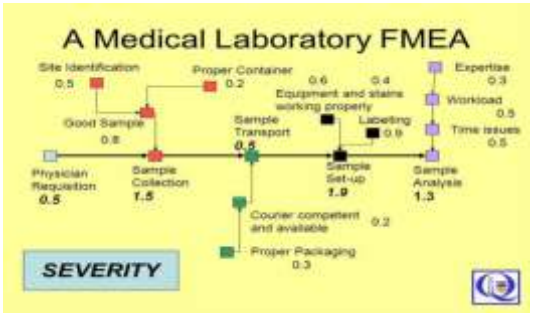
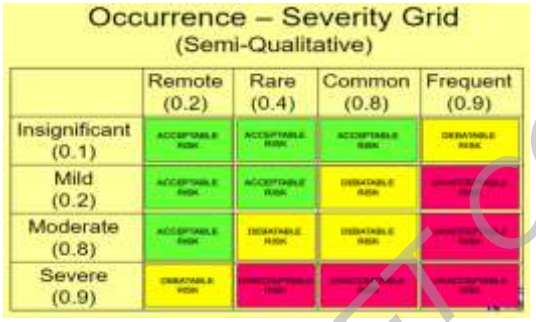
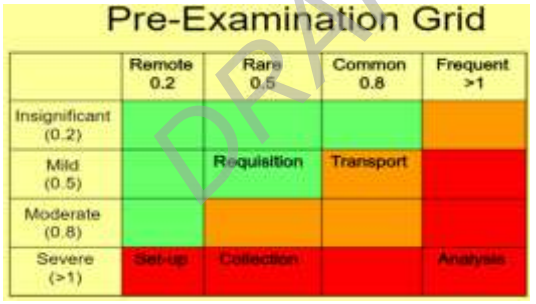
	Each activity of the process is graded from 0-1 as per chances of occurrence
	Each activity of the process is graded from 0-1 as per severity
	A risk prioritization matrix or occurrence severity matric is used to plot the grades
	The data from the pre-analytical process of the above lab is plotted as shown. Areas falling in the red region has to be prioritized for action
	The relationship between Corrective Action, Preventive action, Risk Management and Six Sigma. Both Preventive action plan and Risk management are identical. The latter though is more comprehensive in that, each process needs to be subjected to the analysis systematically

Figure 81: Illustrative examples of FMEA & Risk Analysis

5.5 Pareto Principle

The Pareto principle (also known as the 80–20 rule, the law of the vital few, and the principle of factor sparsity) states that, for many events, roughly 80% of the effects come from 20% of the causes. E.g. 80% of your problems occurs come from 20% of your defects.

It is also called: Pareto diagram, Pareto analysis

Variations: weighted Pareto chart, comparative Pareto charts

A Pareto chart is a bar graph. The lengths of the bars represent frequency or cost (time or money), and are arranged with longest bars on the left and the shortest to the right. In this way the chart visually depicts which situations are more significant.

Many businesses have an easy access to dramatic improvements in profitability by focusing on the most effective areas and eliminating, ignoring, automating, delegating or retraining the rest, as appropriate. What could be the 20% of the issues in communication that results in 80% of the outcomes?

Pareto charts typically show the frequency of occurrence of a variable of interest in different categories arranged in order of descending frequency. The focus is generally on the category that has the highest frequency of occurrence, but in some cases, this typical frequency-based portrait of data is not appropriate. Focusing on the frequency of occurrence of an event is appropriate when the degree of importance is the same for all categories and when the potential for occurrence is the same for all categories. When the frequency approach is not appropriate, the procedure to be used depends on which of these two conditions is not satisfied.

When to Use a Pareto Chart

- When analyzing data about the frequency of problems or causes in a process.
- When there are many problems or causes and you want to focus on the most significant.
- When analyzing broad causes by looking at their specific components.
- When communicating with others about your data.

Pareto Chart Procedure

1. Decide what categories you will use to group items.
2. Decide what measurement is appropriate. Common measurements are frequency, quantity, cost and time.
3. Decide what period of time the Pareto chart will cover: One work cycle? One full day? A week?
4. Collect the data, recording the category each time. (Or assemble data that already exist.)
5. Subtotal the measurements for each category.
6. Determine the appropriate scale for the measurements you have collected. The maximum value will be the largest subtotal from step 5. (If you will do optional steps 8 and 9 below, the maximum value will be the sum of all subtotals from step 5.) Mark the scale on the left side of the chart.

7. Construct and label bars for each category. Place the tallest at the far left, then the next tallest to its right and so on. If there are many categories with small measurements, they can be grouped as "other."

   Steps 8 and 9 are optional but are useful for analysis and communication.

8. Calculate the percentage for each category: the subtotal for that category divided by the total for all categories. Draw a right vertical axis and label it with percentages. Be sure the two scales match: For example, the left measurement that corresponds to one-half should be exactly opposite 50% on the right scale.

9. Calculate and draw cumulative sums: Add the subtotals for the first and second categories, and place a dot above the second bar indicating that sum. To that sum add the subtotal for the third category, and place a dot above the third bar for that new sum. Continue the process for all the bars. Connect the dots, starting at the top of the first bar. The last dot should reach 100 percent on the right scale.

Application in Laboratory Medicine with a dummy example:

An equipment failure in the laboratory is one of the biggest problems, which can occur due to many reasons. Some of the reasons are listed below:

- Lack of regular preventive maintenance
- Environmental factors like dust
- Inadequate calibration
- Poor Handling, like spills
- Electricity Fluctuations

The pareto analysis (as shown in the chart) will help us to understand which reason needs to be addressed first. In the example below, if we address the major reason i.e. lack of regular Preventive maintenance, we can avoid the major instances of Equipment Failure.

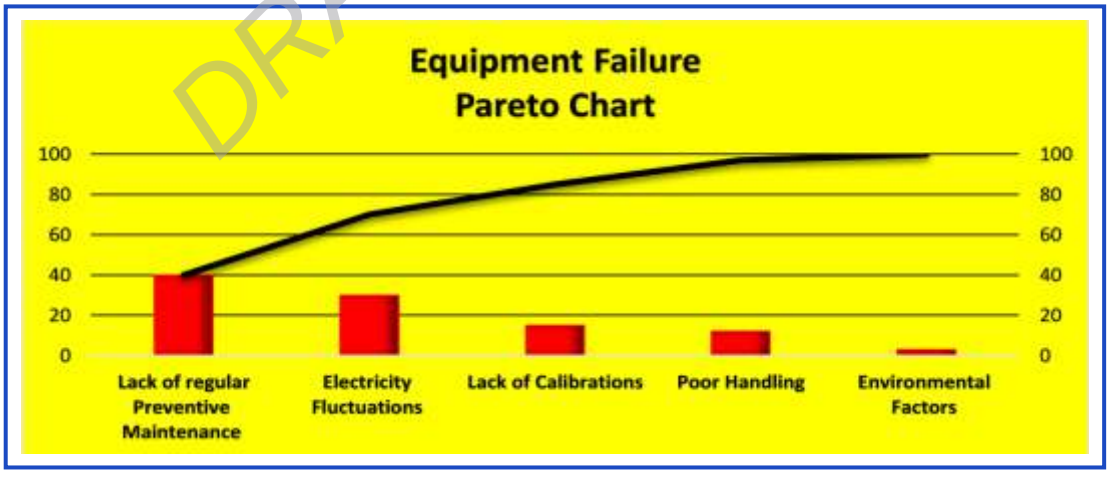


Figure 82: Pareto Chart for equipment failure

Pareto chart can be used anywhere in the laboratory to prioritize the incidents and address them. Data serves as the key factor in this.

Critical decisions on lab activities sometimes are based trends, which often are presented without a statistical analysis. Those responsible for decision making may be left wondering whether these apparent trends represent only chance variation. Trend analysis is based on the idea that what has happened in the past will happen in the future.

5.6 Trend Analysis

Why Do Trend Analysis

- Comparing one time period to another time period
- Comparing one group to another
- Making future projections
- Comparing with other organizations

Applications in Laboratory Medicine with an example

- Consumptions of Reagents over a period (daily, weekly, monthly, and annually)
- Peer group analysis (EQAS reporting) – comparison with many labs
- To observe patient load in laboratory department wise, test wise etc.
- To see the trend of CV of various parameters to see the quality

How to do trend Analysis

- It is one of the easy and simple techniques used. Data is collected over a period of time and is plotted on charts. The trends are observed to take a decision accordingly.

Application of Trend analysis in laboratory, single analyte

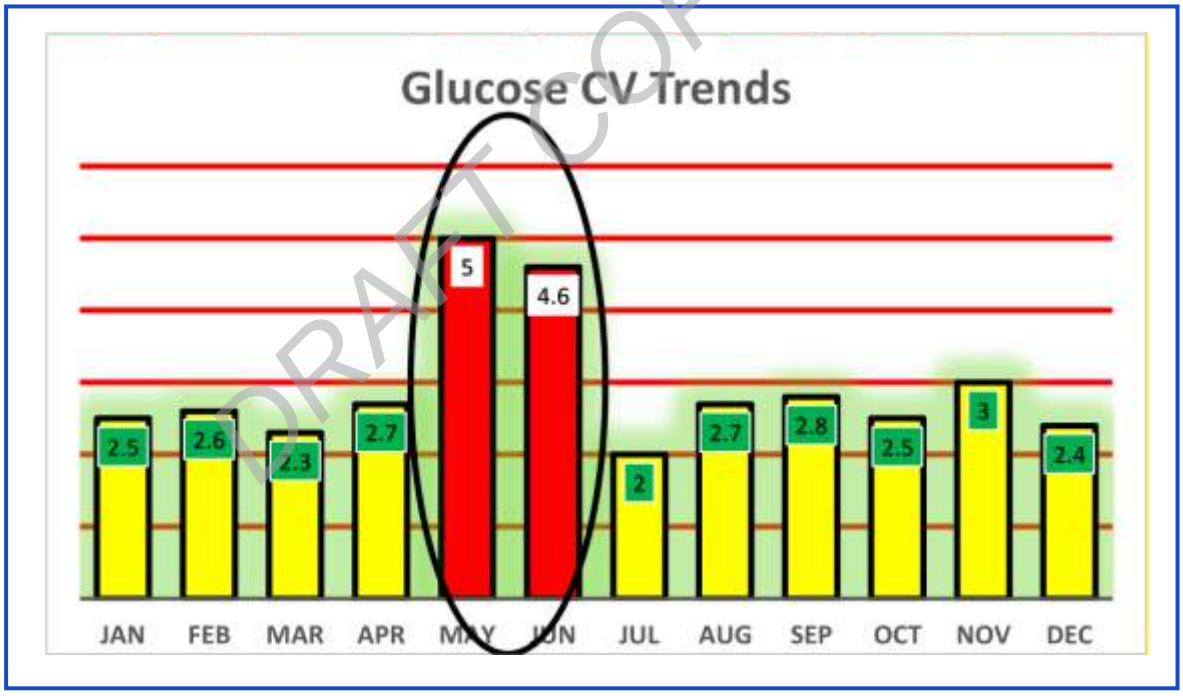


Figure 83: Graphical representation of Trend Analysis of single parameter over a period of one year

CV trend analysis, multiple analytes, multiple months.

The figure below shows a set of data of CVs of analytes which are arranged analyte-wise and month-wise. Consistently high CVs in Creatinine are seen, pointing towards a consistent imprecision, possibly implying a reagent defect. A root cause analysis needs to be done. A sudden increase in the CVs is seen in April pointing to some shift in the equipment performance and warranting root cause analysis. A room temperature rise, an equipment malfunction such as probe issues, storage temperature of multiple reagents pointing a refrigerator malfunction etc. need to be considered.

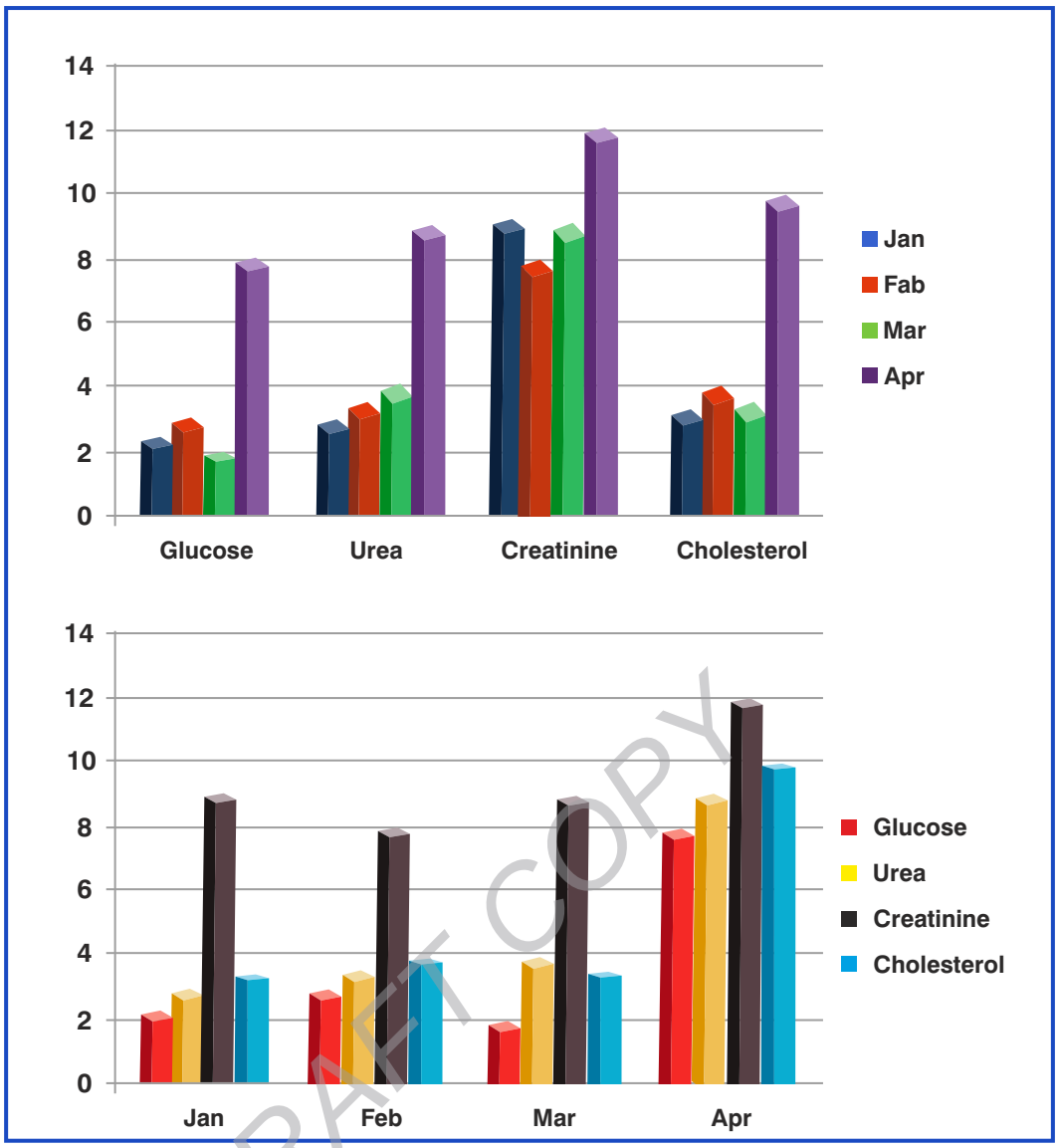


Figure 84: Graphical representation of Trend Analysis of multiple parameters over a period of time, both parameter wise and month wise

5.7 Root cause analysis (RCA) & Cause & Effect Analysis

- Root Cause Analysis (RCA) is a structured method used to analyze incidents and adverse events. Initially developed to analyze industrial accidents, RCA is now widely deployed as an error analysis tool in health care.
- A root cause is an initiating cause of either a condition or a causal chain that leads to an outcome or effect of interest. Commonly, root cause is used to describe the depth in the causal chain where an intervention could reasonably be implemented to improve performance or prevent an undesirable outcome.

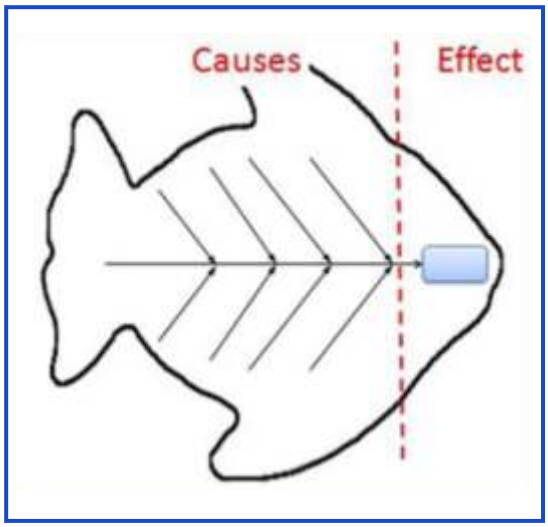


Figure 85: Fishbone diagram

As example, imagine a day in the lab with the very high workload of samples, and the major equipment broke down. An investigation into the machine that stopped because it was overloaded and the fuse blew. Investigation shows that the machine overloaded because it had a bearing that wasn't being sufficiently lubricated. The investigation proceeds further and finds that the automatic lubrication mechanism had a pump which was not pumping sufficiently, hence the lack of lubrication. Investigation of the pump shows that it has a worn shaft. Investigation of why the shaft was worn discovers that there isn't an adequate mechanism to prevent metal scrap getting into the pump. This enabled scrap to get into the pump, and damage it. The root cause of the problem is therefore that metal scrap can contaminate the lubrication system. Fixing this problem ought to prevent the whole sequence of events recurring. Compare this with an investigation that does not find the root cause: replacing the fuse, the bearing, or the lubrication pump will probably allow the machine to go back into operation for a while. But there is a risk that the problem will simply recur, until the root cause is dealt with.

In India, one of the causes for recurrent equipment breakdown is dust and lack of proper maintenance.

The primary aim of root cause analysis is:

- To identify the factors that resulted in the nature, the magnitude, the location, and the timing of the harmful outcomes (consequences) of one or more past events; to determine what behaviors, actions, inactions, or conditions need to be changed; to prevent recurrence of similar harmful outcomes; and to identify lessons that may promote the achievement of better consequences. ("Success" is defined as the near-certain prevention of recurrence.)
- To be effective, root cause analysis must be performed systematically, usually as part of an investigation, with conclusions and root causes that are identified backed up by documented evidence. A team effort is typically required.
- There may be more than one root cause for an event or a problem, wherefore the difficult part is demonstrating the persistence and sustaining the effort required to determine them.
- The purpose of identifying all solutions to a problem is to prevent recurrence at lowest cost in the simplest way. If there are alternatives that are equally effective, then the simplest or lowest cost approach is preferred.
- The root causes identified will depend on the way in which the problem or event is defined. Effective problem statements and event descriptions (as failures, for example) are helpful and usually required to ensure the execution of appropriate analyses.

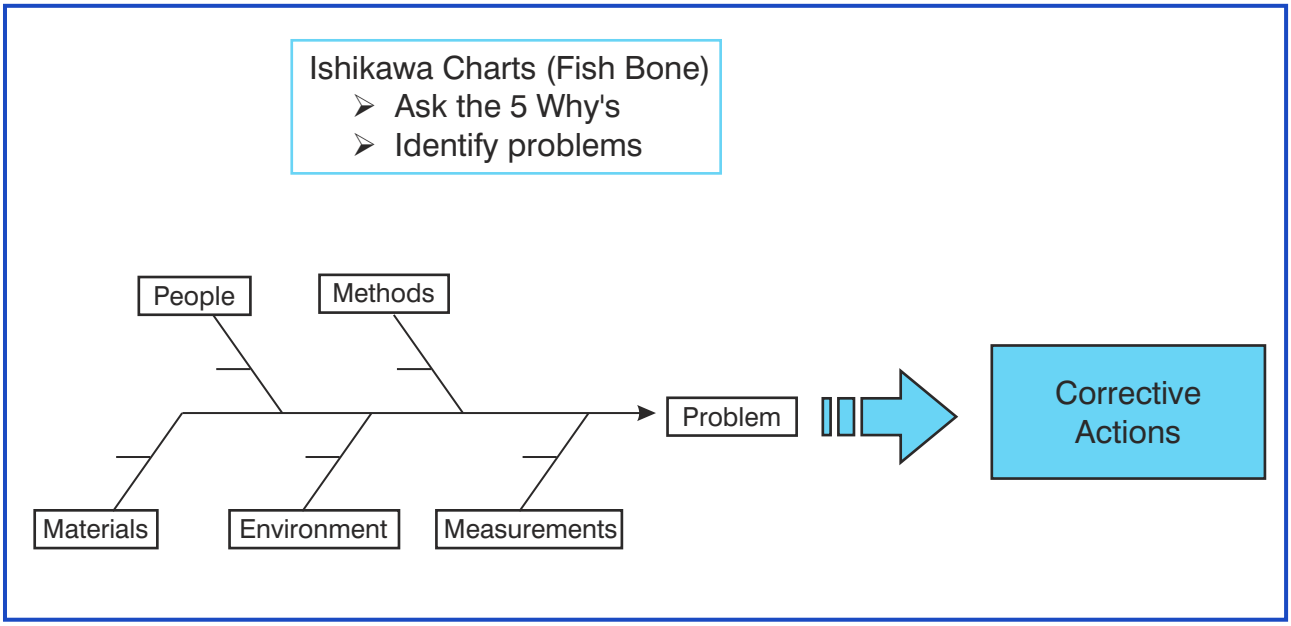


Figure 86: Using a Fishbone tool

Four Major Steps in RCA

The RCA is a four-step process involving the following:

1. Data collection.
2. Causal factor charting
3. Root cause identification.
4. Recommendation generation and implementation.

Value Stream Mapping

Value Stream Mapping (VSM) is following a product's production path from beginning to end. In the case of a lab, it is a sample. Wasteful or non-value adding aspects are: confusion, unnecessary motion/conveyance (physical movement required to get a simple task accomplished and to move people and products from place to place), waiting, over-processing (doing more activities than is necessary to complete a piece of work), inventory issues (obsolete, duplicated, unnecessary, or missed items), defects (errors) and overproduction (an example, redundant paperwork). All these wasteful activities can occur along the sample path in a lab.

A proper Value Stream Mapping along the sample path creates value, eliminates waste, reduces lead time and in turn reduces, total costs. The following are a few examples of the results of a VSM in a lab, increasing productivity

Reducing analytical batch sizes and increasing the frequency of analyses
Middleware to interface instrumentation with the LIMS
Staggering shifts
Cross training analysts for reporting
Automation of manual analyses

So it is vital that the staff and management of a laboratories undertake VSM to enhance the performance and avoid mistakes.

The above processes will enable the laboratory to identify bottlenecks and avoid & mitigate risks. Though many of the techniques seem self-evident and easily doable, unless the laboratory invests time and efforts into practicing these, several hidden problems will never come into view resulting in unforeseen breakdowns and risks jeopardizing the safety of the patients, reports, and staff.

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Glossary

Risk assessment	:	Identifying potential failure modes, determining severity of consequences, identifying existing controls, determining probabilities of occurrence and detection, and evaluating risks to identify essential control points.
Accuracy	:	The closeness of a measurement to its true value.
Across Material	:	Using the different materials of QC (Levels) to assess the performance at the same time
Across the runs	:	Using the same materials to assess the performance at difference times
Bias	:	Difference between the expectation of the test results and an accepted reference value.
Algorithm	:	A process or set of rules to be followed in calculations or other problem-solving operations, especially by a computer
Calibrators	:	Solutions with specified defined concentrations that are used to set or calibrate an instrument, kit or system before testing is begun. Calibrators are often provided by the manufacturer of an instrument.
Carryover	:	The discrete amount of reagent or analyte carried by the measuring system from one test into subsequent test(s), thereby erroneously affecting test results.
Coefficient of Variation (CV)	:	The standard deviation (SD) expressed as a percentage of the mean.
Consensus (Within & out-with)	:	Establishing characteristic values for components or properties of a material, for quality control. Within and out-with are whether the observed values are within the limits of the established values said above.
Control chart	:	A chart with upper and lower control limits on which values of some statistical measure for a series of samples or subgroups are plotted. The chart frequently shows a central line to help detect a trend of plotted values toward either control limit.
Control material	:	Substance, material or article used to verify the performance characteristics of an in vitro diagnostic medical device.
Current Lot	:	The lot of QC which is being used at the moment. Any new lot of QC needs to be validated simultaneously while the current lot is used for monitoring the performance of analytical system.
Error	:	A deviation from truth, accuracy or correctness; a mistake; a failure of a planned action to be completed as intended, or the use of a wrong plan to achieve an aim.

External quality assessment (EQA)	:	A system for objectively checking the laboratory's performance using an external agency or facility.
Gaussian Distribution	:	A property exhibited by appropriately preserved biological material, on repeated analysis, whereby the data points show normal distribution i.e. 68-95-99 rule.
Harmonization	:	A method of assuring comparability of tests done using different mechanism or machines, which may employ different methods and have different traceability of calibrators.
Lyophilized	:	Freeze dried material which require reconstitution before use.
Manufacturer's Mean	:	A stated mean for a control material as defined by the manufacturer of the material. This requires to be verified in the lab before being used to verify the performance of an analytical system
Measurand	:	The analyte being measured by the measuring system
Observed Mean	:	The mean observed by the lab while running a QC for a defined period of time. Also called obtained/lab mean.
Performance Evaluation	:	An ongoing process whereby the system is checked for fitness for use.
Precision	:	Closeness of agreement between quantity values obtained by replicate measurements of a quantity, under specified conditions. See Quantitative examination.
Process control	:	Concerns monitoring all operations of the laboratory.
Qualitative	:	Relating to, measuring, or measured by the quality of something rather than its quantity
Quality system essentials	:	The necessary infrastructure or foundational building blocks in any organization that need to be in place and functioning effectively in order to support the organization's work operations so that they proceed smoothly. See Quality management. CLSI developed the quality management framework and organized the topics as the "12 Quality System Essentials" based on both ISO 15189 and CLSI GP26-A3 documents.
Quantitative	:	relating to, measuring, or measured by the quantity of something rather than its quality
Reference Interval	:	It is the range of test values expected for a designated population in which 95% of the individuals are presumed to be healthy (or normal). In some analytes reference interval have been replaced by decision limits established by international consensus. Also called BRI (Biological reference interval)/BRR (Biological reference range).
Quality Specification	:	Defining the analytical goals of a laboratory. This is the responsibility of the laboratory head. Also termed as a Quality requirements.

Risk management	:	The identification, analysis and economic control of those risks which can threaten the assets or earnings of an enterprise.
Root Cause	:	A factor that caused a nonconformity and should be permanently eliminated through process improvement.
Rule Selection	:	Deciding on using single rule or multi rules and which rules to use depending on the performance of the analyte.
Semi quantitative examination	:	Test whose results are expressed as a rough estimate of how much of the measured substance is present.
Sensitivity	:	Sensitivity is the lowest concentration of an analyte that can be measured. This is also LoD/LoQ
Specificity	:	Artifactual increase or decrease of quantity of analyte due to the presence of any interfering substance(s).
Statistical tools	:	Methods and techniques used to generate, analyze, interpret and present data.
Turnaround time	:	Length of time that a sample's final result may be issued to the ordering physician.
Validation	:	Confirmation, through provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.
Medical Decision Points	:	Values of analytes which is significant clinically. May be high, low or normal. Other terms used are Medical Decision Values (MDV), Clinical Decision points (CDP), Clinical Decision Values (CDV).
Verification	:	Confirmation, through provision of objective evidence, that specified requirements have been fulfilled.
Within Material	:	Using the same QC material across runs.
Within Run	:	Using the different QC material in a run.

Abbreviations

ADA	:	American Diabetes Association
AMR	:	Analytical Measurement range
AON	:	Average of Normal
ASC	:	Atypical Squamous cells
BRR	:	Biological Reference Range
BRI	:	Biological Reference Interval
BV	:	Biological Variations
CCV	:	Chosen Coefficient of Variation
CLIA	:	Clinical Laboratory Improvement Amendments
CLSI	:	Clinical and Laboratory Standards Institute
CV	:	Coefficient of Variation
CVI	:	Coefficient of Variation Index
DV	:	Designated Value
ELISA	:	enzyme-linked immunosorbent assay
EQAS	:	External Quality Assurance Scheme
FDA	:	Food & Drug Administration
FIFO	:	First in First Out
FMEA	:	Failure Mode Effect Analysis
IFU	:	Instruction for Use
IHC	:	Immuno- Histo Chemistry
ILC	:	Inter-laboratory Comparison
INR	:	International Normalized Ratio
IQC	:	Internal Quality Control
ISI	:	International Standardization Index
ISO	:	The International organization for Standardization
JIT	:	Just in Time
LJ	:	Levey Jennings
LoD	:	Limit of Detection
LoQ	:	Limit of Quantification

LQMS	:	Laboratory Quality Management System
MDP	:	Medical Decision Point
ME	:	Method Evaluation
MNPT	:	Mean Normal Prothrombin Time
MU	:	Measurement Uncertainty
PDCA	:	Plan, Do, Check, Act (quality improvement tool)
Ped	:	Percent error detection
Pfr	:	Percent false rejection
PT	:	Prothrombin Time
PT	:	Proficiency Testing
QC	:	quality control
RCA	:	Root Cause Analysis
RDT	:	Rapid Diagnostic Tests
RE	:	Random Error
RPM	:	Revaluation Per minute
SD	:	Standard Deviation
SDI	:	Standard Deviation Index
SE	:	Systematic Error
SEc	:	Critical Systematic Error
SQC	:	Statistical Quality Control
TE	:	Total Error
TEa	:	Total Allowable Error (Also called ATE; Allowable Total Error)
TQM	:	Total Quality Management
URS	:	User Specification Requirement
VIS	:	Variance Index Score
VSM	:	Value Streaming Mapping
TDPA	:	Target Deviation for Performance Assessment
SDPA	:	Standard Deviation for Performance Assessment

Annexure

Annexure 1	**Job-Aids** A. Equations B. Creating a L-J Chart C. Navigating the Westgard Internet Site (www.westgard.com) D. Steps for Developing a QC Strategy E. General Guidelines for Proficiency Testing
Annexure 2	**Total Allowable Error Limits** A. BV Desirables B. CLIA Limits C. Recommended TEa Limits (Sun Diagnostic)
Annexure 3	**Medical Decision Points** (Extracts from Westgard site)
Annexure 4	**Sigma-Metrics QC Selection Tool** A. Sigma-Metrics QC Selection Tool for 2 Levels Control B. Sigma-Metrics QC Selection Tool for 3 Levels Control
Annexure 5	**Frequency and Scope of Testing: Commonly used EQAS Schemes**
Annexure 6	**Corrective Action Formats for IQC & EQA** A. Corrective Action formats for IQC B. Corrective Action formats for EQA (PT Failure Checklist)
Annexure 7	**Evaluation Summary Report**
Annexure 8	**Worksheets**

Job-Aids

Annexure 1

A. Equations

$$\bar{x} = \frac{\Sigma x_i}{n}$$

$$SD = \sqrt{\frac{\Sigma(X_i - \bar{x})^2}{n-1}}$$

$$CV\% = (SD / \bar{x}) * 100\%$$

$$\text{Z-score} \quad \frac{\text{Mean} - \text{Target}}{SD}$$

$$\text{Bias} = \bar{x} - \text{True Value}$$

$$\text{Absolute Bias} = |\bar{x} - \text{True Value}|$$

$$\%\ \text{bias} = (\text{bias/target value}) * 100\%$$

$$TE = |\bar{x} - \text{True Value}| + (\text{z factor} * SD) = |\text{bias}| + (\text{z factor} * SD)$$

$|\bar{x} - \text{True Value}| + 2\ SD$ ease for computation

$|\bar{x} - \text{True Value}| + 1.96\ SD$ 97.5% of the population of data points included in the estimation of total error

$|\bar{x} - \text{True Value}| + 1.65\ SD$ 95% of the population of data points included in the estimation of total error

$$\%\ TE = \%\ \text{bias} + (\text{z factor} * CV\%) \cong (TE_{\text{in units}} / \text{Target Value}_{\text{in units}}) * 100\%$$

% bias + 2CV%	ease for computation
% bias + 1.96 CV%	97.5% of the population of data points included in the estimation of total error
% bias + 1.65 CV%	95% of the population of data points included in the estimation of total error

$TE < TE_A$

$Sigma = [(TEa - |bias_{obs}|)/SD_{obs}]$

$\Delta SEc = [(TEa - |bias_{obs}|)/SD_{obs}] - z\ factor = Sigma - z\ factor$

Sigma – 1.65 =	ΔSEc value used by Dr. Westgard where 5% of the population of data points exceed TE_A limits

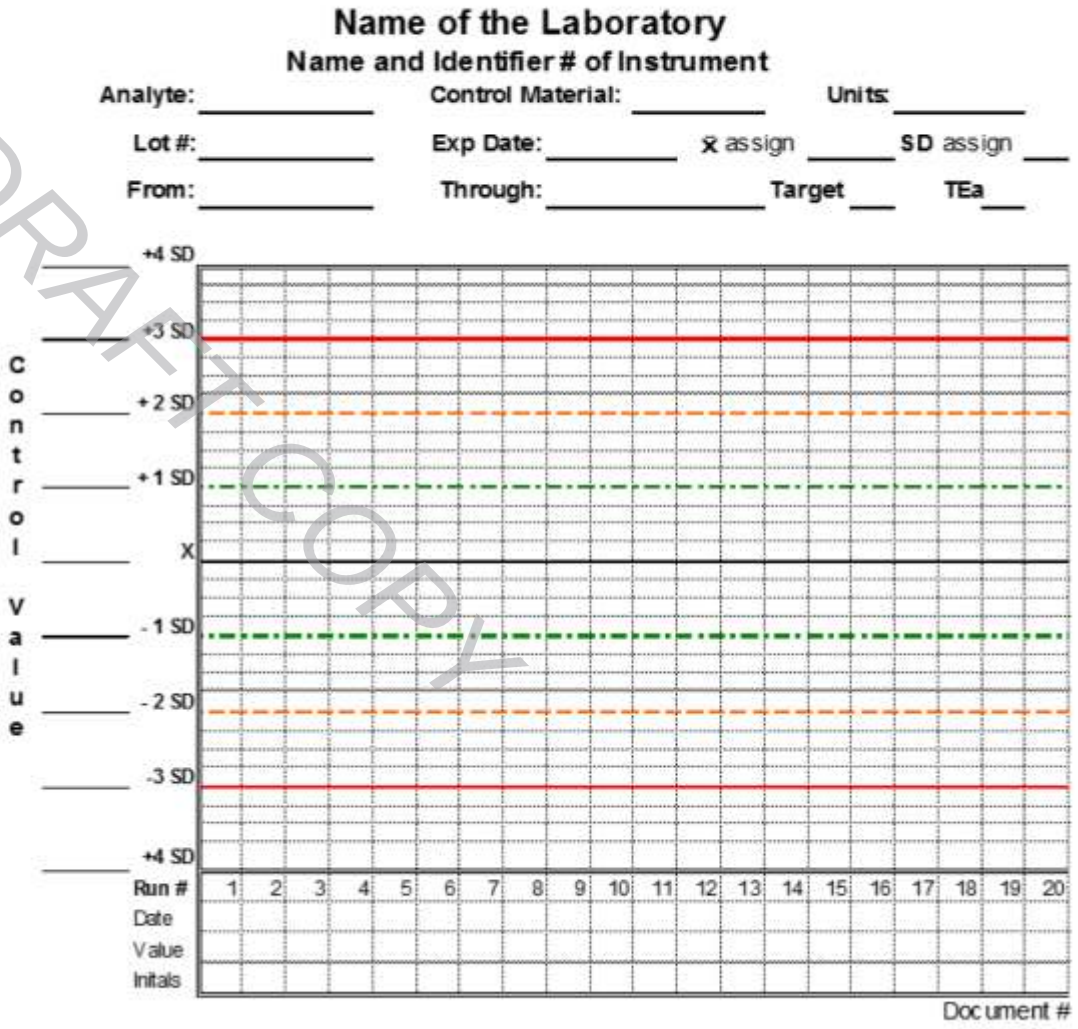
$Sigma = \Delta SEc + 1.65$

$SDI = (\bar{x}\ lab - \bar{x}\ group)/\ SDgroup$

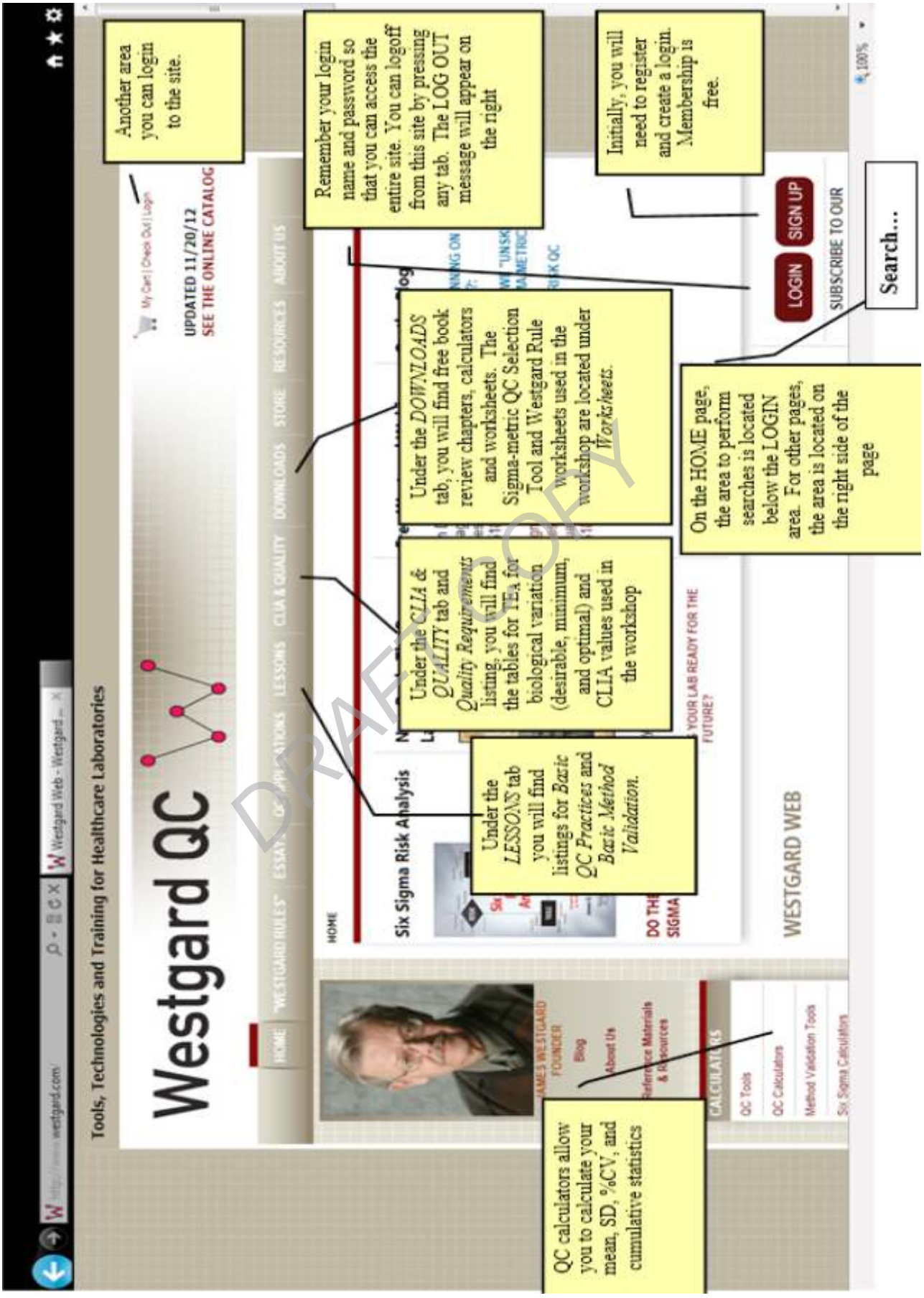
CVI (CVR) = within lab CV/peer group CV

Creating a L-J Chart

1. Label the chart
   - Name of the laboratory
   - Name of the Instrument and Identifier #
   - Name of Test
   - Units
   - Name & expiration date of the control material
   - Assigned mean and standard deviation used on the chart
   - Acceptable control limits
   - Time period covered by the chart
2. Label the X-axis in terms of time period used or run
3. Scale the X-axis into evenly sized increments numbering sequentially
4. Label the Y-axis Control Value and for *X, ±1 SD, ±2 SD, ±3 SD, ±4 SD*
5. Scale the Y-axis from lowest to highest expected control values as follows using the assigned mean and standard deviation (SD) so that the mean is located at the center of your graph:
   - Subtract the SD from the mean; this is the -1SD
   - Add the SD to the mean; this is the +1SD
   - Multiply the SD by 2, and then subtract that value from the mean. This is the -2SD
   - Multiply the SD by 2 and then add that value to the mean. This is the +2SD
   - Repeat this process for ± 3SD, ± 4SD using a factor of 3 and 4 respectively.
6. Write the values obtained for *X, ±1 SD, ±2 SD, ±3 SD, ±4 SD* next to the correct label on the chart.
7. Draw lines for mean and SDs
8. Begin plotting analyzed QC results.



C. Navigating the Westgard Internet Site (www.westgard.com)



D. Steps for Developing a QC Strategy

1. **Select a test.**
2. **Select appropriate control materials.**
3. **Determine your TE_A limits**
   a. Select the TEA for the test; note the resources used for the selection.
   b. Select the Target Value (Clinical Decision Concentration) for each control; note the resource used for the selection.
   c. Calculate the TEA in units.
4. **Determine current method performance**
   a. Calculate the current method's mean from a stable system for each control.
   b. Calculate the current method's SD from a stable system for each control.
   c. Calculate the SEc and Sigma-metric for each control; if SEc is zero or a negative number, then your TE $\geqslant TE_A$. Stop reporting patient results immediately, verify your four Key Numbers of Quality, and fix the problem(s)
5. **Select appropriate control rules**
   a. Choose the appropriate Sigma-metrics QC Selection Tool for the number of controls used for the test.
   b. Locate the Sigma-metric value on the Sigma-scale (scale at the top of the X-axis).
   c. Validate the Sigma-metric against the SEc scale (scale at the bottom of the X-axis).
   d. Draw a vertical line from the Sigma-metric value to the SEc value.
   e. Assess probability of error rejection where the Sigma line intersects with the QC rule power curve.
   f. Identify candidate QC rules in which Ped is $\geqslant$ 0.90 (90%).
   g. Assess false rejection rates of candidate QC rules from the table [$\leqslant$ 0.05 (5%)].
   h. Select the appropriate QC rule and total number of control measurements (N) that provide the lowest cost and are easiest to implement.
6. **On-going monitoring of QC**
   a. Create the QC chart.
   b. Determine how often a supervisor will review the QC chart, depending on the SEc or Sigma-metric.
   c. Initiate corrective action if SEc and Sigma are low.
   d. Develop a standardized process to investigate QC rule violations from daily, summary, and peer-reviewed QC data.
   e. Monitor the accuracy, precision, SEc, and Sigma at least on a monthly basis.
   f. Take corrective actions as needed; continue to target poorly-performing analytical systems.
7. **Document this entire process.**
8. **Educate the analytical staff.**
9. **Communicate with upper management regarding the laboratory's needs for a complete QC process.**

E. General Guidelines for Proficiency Testing

Laboratory proficiency testing (PT) is an essential element of laboratory quality assurance. Proficiency testing is an independent and unbiased assessment that evaluates the laboratory's ability to produce correct answers. Proficiency testing provides an assessment of the validity of testing in your laboratory.

Handling Your PT Survey

Pre-analytical

- Note the date of receipt for your shipment
- Immediately inspect and reconcile the contents of your shipment with the accompanying paperwork
- Are all required specimens available?
- Is the quality and appearance of the specimens acceptable?
  - Store the shipment properly
  - Note due date of results
  - Reconstitute specimens with volumetric pipettes and correct diluent
  - Mix samples well before analyzing

Analytical

- Analyze specimens at correct temperature. If shipment was stored in the refrigerator, specimens may need to come to room temperature before testing.
- Always refer to your survey instructions for storage and specimen handling.
- Analyze PT specimens in the same fashion as patient specimens.
- Do not refer any PT samples to another laboratory, even if your instrument is non-functioning or is part of your testing algorithm.
- Rotate testing responsibility for PT specimens between all laboratory personnel that are routinely performing the analysis in your laboratory.
- Perform PT analysis well before due date of results.

Post-Analytical

- Assure that your laboratory's results are reported according to the PT provider's instructions.
- Ensure the proper method and instrument code are recorded for each test so that you are part of the correct peer group.
- If test not performed is the correct answer because of equipment issues, then indicate this on the form.
- If the result obtained requires additional testing per your laboratory's algorithm, then indicate on the form to be sent to a reference laboratory or further testing required, but do not actually send the PT sample to another laboratory.
- Review results for clerical errors on answer sheet, including decimal point placement.

- Retain a copy of answer sheet for your records. Attach all raw data and the instrument print-out to the answer sheet.
- If possible, retain specimens in freezer for confirmatory testing if needed.
- If you use the PT sample materials to cross-check other instrument or methods, or as part of your competency training program, then be absolutely sure the PT results are submitted to the PT provider before starting these activities.

Receipt of Results

- Review your results with your peer grouping.
- Investigate all unacceptable grades.
- Have the Laboratory Director and Supervisor review, and sign and date results.
- Review results with testing personnel. Retain a copy for competency assessment and place into personnel record.
- Investigate any failed responses and complete an EQA Failure Checklist assessment.
- Follow-up with remedial actions if indicated.

Annexure 2

Total Allowable Error Limits

A. BV

Desirable Specification: Page1 (Sample)

Biological Variation Values

Desirable Analytical Quality Specifications for Imprecision, Bias and Total Error Upon Biological Variation

The following values are provided as a service to Bio-Rad Customers and are based upon desirable performance. The values are derived from Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Mininchela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress" Scand J Clin Lab Invest 1999;59:491-500. These values are updated/ modified with the most recent specifications made available in **2014**. **•(denotes updated values)**

S = serum; U = urine; P = plasma; B = blood
CV_w = within-subject biological variation; CV_b = between-subject biological variation; Imp = imprecision; TE_a = total allowable error

		BIOLOGICAL VARIATION		DESIRABLE SPECIFICATIONS			
	ANALYTE	CV_w	CV_b	Imp (%)	Bias (%)	TE_a (%) p=0.05	TE_a (%) p=0.01
S	11-Deoxycortisol	21.3	31.5	10.7	9.5	27.1	34.3
S	17-Hydroxyprogesterone	19.6	50.4	9.8	13.5	29.7	36.4
U	5-HIAA concentration, 24 h	20.3	33.2	10.2	9.7	26.5	33.4
S	5'Nucleotidase	23.2	19.9	11.6	7.6	26.8	34.7
S	α1-Acid glycoprotein	11.3	24.9	5.7	6.8	16.2	20.0
S	α1-Antitrypsin	5.9	16.3	3.0	4.3	9.2	11.2
S	α1-Globulin	11.4	22.6	5.7	6.3	15.7	19.6
S	α2-Globulins	10.3	12.7	5.2	4.1	12.6	16.1
U	α1-Microglobulin	33.0	58.0	16.5	16.7	43.9	55.1
S	α2-Macroglobulin	3.4	18.7	1.7	4.8	7.6	8.7
P	α-Aminobutyric Acid (AABA)	24.7	32.3	12.4	10.2	30.5	38.9
S	α-Amylase	8.7	28.3	4.4	7.4	14.6	17.5
U	α-Amylase	94.0	46.0	47.0	26.2	103.7	135.7
S	α-Amylase, pacreatic	11.7	29.9	5.9	8.0	17.7	21.7
S	Acid phosphatase (ACP)	8.9	8.0	4.5	3.0	10.3	13.4
P	Activated partial thromboplastin time	2.7	8.6	1.4	2.3	4.5	5.4
S	Adenosine Deaminase (ADA)	11.7	25.5	5.9	7.0	16.7	20.6
P	Adiponectin	18.8	51.2	9.4	13.6	29.1	35.5
S	AFP	12.2	45.6	6.1	11.8	21.9	26.0
P	Alanine	14.7	55.8	7.4	14.4	26.6	31.6
S	* Alanine aminotransferase	19.4	41.6	9.7	11.5	27.5	34.1
S	* Albumin	3.2	4.75	1.6	1.4	4.1	5.2
U	* Albumin	35	35	17.5	12.4	41.2	53.1
U	Albumin: Creatinine Ratio	30.5	32.5	15.3	11.1	36.3	46.7
S	Aldosterone	29.4	40.1	14.7	12.4	36.7	46.7
U	* Aldosterone concentration, 24 h	39.4	40.1	19.7	14.1	46.6	60.0
S	* Alkaline phosphatase	6.45	26.1	3.2	6.7	12.0	14.2
S	Alkaline phosphatase, bone	6.2	37.4	3.1	9.5	14.6	16.7
U	* Aminolevulinic Acid	16	27	8.0	7.8	21.0	26.5
U	Ammonia output, 24 h	24.7	27.3	12.4	9.2	29.6	38.0
S	* Androstendione	15.8	38.8	7.9	10.5	23.5	28.9
S	Anion Gap	9.5	10.1	4.8	3.5	11.3	14.5
P	Antiplasmin activity	6.2		3.1			
P	Antithrombin III	5.2	15.3	2.6	4.0	8.3	10.1
S	Apolipoprotein A1	6.5	13.4	3.3	3.7	9.1	11.3
S	Apolipoprotein B	6.9	22.8	3.5	6.0	11.6	14.0
S	Ascorbic Acid (Vitamin C)	26.0	31.0	13.0	10.1	31.6	40.4
P	* Ascorbic Acid (Vitamin C)	20	21	10.0	7.3	23.8	30.6

B. CLIA Limits

Analyte or Test	CLIA Criteria for Acceptable Performance
Alcohol, Blood	± 25%
Alanine Aminotransferase (ALT/SGPT)	± 20%
Albumin	± 10%
Alkaline Phosphatase	± 30%
Alpha-1 Antitrypsin	Target value ± 3 SD
Alpha-Fetoprotein (Tumor Marker) AFP	Target value ± 3 SD
Amylase	± 30%
Antinuclear Antibody	Target value ± 2 dilutions or positive/ negative
Antistreptolysin O	Target value ± 2 dilutions or positive/ negative
Anti-Human Immunodeficiency Virus	Reactive or nonreactive
Aspartate Aminotrasnferase (AST/SGOT)	± 20%
Bilirubin, Total	Target value ± 20% or ± 0.4 mg/dL (greater)
Calcium, Total	Target value ± 1.0 mg/dL
Carbamazepine	± 25%
Cell Identification	90% or greater consensus on identification
Chloride	± 5%
Cholesterol, High Density Lipoprotein	± 30%
Cholesterol, Total	± 10%
Complement C3	Target value ± 3 SD
Complement C3C	Target value ± 3 SD
Complement C4	Target value + 3 SD
Cortisol	± 25%
Creatine Kinase	± 30%
Creatine Kinase CK-MB	Target value ± 3 SD or presence/ absence
Creatinine	Target value ± 15% or ± 0.3 mg/dL (greater)
Digoxin	Target value ± 20% or ± 0.2 ng/mL (greater)
Erythrocyte Count RBC	± 6%
Ethosuximide	± 20%
Fibrinogen	± 20%
Free Thyroxine Free T4	Target value ± 3 SD
Gentamicin	± 25%
Glucose	Target value ± 10% or ± 6 mg/dL (greater)
Hematocrit (Excluding Spun Hematocrits) HCT	± 6%
Hemoglobin Hgb, Total	± 7%
Hepatitis (HbsAg, anti-HBc, HbeAg)	Reactive (positive) or nonreactive (negative)
Human Chorionic Gonadotropin Beta	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Intact	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Qualitative	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Total	Target value ± 3 SD or positive/ negative
IgA	Target value ± 3 SD
IgE	Target value ± 3 SD
IgG	± 25%
IgM	Target value ± 3 SD
Infectious Mononucleotides	Target value ± 2 dilutions or positive/ negative
Iron, Total	± 20%

Analyte or Test	CLIA Criteria for Acceptable Performance
Alcohol, Blood	± 25%
Alanine Aminotransferase (ALT/SGPT)	± 20%
Albumin	± 10%
Alkaline Phosphatase	± 30%
Alpha-1 Antitrypsin	Target value ± 3 SD
Alpha-Fetoprotein (Tumor Marker) AFP	Target value ± 3 SD
Amylase	± 30%
Antinuclear Antibody	Target value ± 2 dilutions or positive/ negative
Antistreptolysin O	Target value ± 2 dilutions or positive/ negative
Anti-Human Immunodeficiency Virus	Reactive or nonreactive
Aspartate Aminotrasnferase (AST/SGOT)	± 20%
Bilirubin, Total	Target value ± 20% or ± 0.4 mg/dL (greater)
Calcium, Total	Target value ± 1.0 mg/dL.
Carbamazepine	± 25%
Cell Identification	90% or greater consensus on identification
Chloride	± 5%
Cholesterol, High Density Lipoprotein	± 30%
Cholesterol, Total	± 10%
Complement C3	Target value ± 3 SD
Complement C3C	Target value ± 3 SD
Complement C4	Target value ± 3 SD
Cortisol	± 25%
Creatine Kinase	± 30%
Creatine Kinase CK-MB	Target value ± 3 SD or presence/ absence
Creatinine	Target value ± 15% or ± 0.3 mg/dL (greater)
Digoxin	Target value ± 20% or ± 0.2 ng/mL (greater)
Erythrocyte Count RBC	± 6%
Ethosuximide	± 20%
Fibrinogen	± 20%
Free Thyroxine Free T4	Target value ± 3 SD
Gentamicin	± 25%
Glucose	Target value ± 10% or ± 6 mg/dL (greater)
Hematocrit (Excluding Spun Hematocrits) HCT	± 6%
Hemoglobin Hgb, Total	± 7%
Hepatitis (HbsAg, anti-HBc, HbeAg)	Reactive (positive) or nonreactive (negative)
Human Chorionic Gonadotropin Beta	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Intact	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Qualitative	Target value ± 3 SD or positive/ negative
Human Chorionic Gonadotropin Total	Target value ± 3 SD or positive/ negative
IgA	Target value ± 3 SD
IgE	Target value ± 3 SD
IgG	± 25%
IgM	Target value ± 3 SD
Infectious Mononucleotides	Target value ± 2 dilutions or positive/ negative
Iron, Total	± 20%

C. Recommended TEa Limits (Sun Diagnostic)

ASSURANCE™ INTERFERENCE TEST KIT

RECOMMENDED TOTAL ALLOWABLE ERROR LIMITS

Sun Diagnostics

Sun Diagnostics has compiled this list of Total Allowable Error limits for a variety of laboratory tests as defined by CLIA or other industry standards. This list is intended as a reference only. Laboratories are responsible for setting their own performance criteria.

Chemistry Analyte	Limit	Source
Albumin (ALB)	± 10%	CLIA
Alkaline Phosphatase (ALP)	± 30%	CLIA
Alanine Aminotransferase (ALT)	± 20%	CLIA
Amylase (AMY)	± 30%	CLIA
Aspartate Aminotransferase (AST)	± 20%	CLIA
Bilirubin, Total (TBILI)	± 0.4 mg/dL or 20% (greater)	CLIA
Calcium (CA)	± 1.0 mg/dL	CLIA
Cholesterol, Total (CHOL)	± 10% ± 9%	CLIA NCEP
HDL Cholesterol (HDL-C)	± 30% ± 13%	CLIA NCEP
LDL Cholesterol (LDL-C)	± 12%	NCEP
Chloride (CL)	± 5%	CLIA
Creatine Kinase (CK)	± 30%	CLIA
Creatinine (CREA)	± 0.3 mg/dL or 15% (greater) ± 7.6% (desirable), ± 11.4% (minimum)	CLIA NKDEP
Glucose (GLU)	± 6 mg/dL or 10% (greater)	CLIA
Hemoglobin A1c (HbA1c)	± 6%	NGSP
IRON (FE)	± 20%	CLIA
Lactate Dehydrogenase (LDH)	± 20%	CLIA
Magnesium (MG)	± 25%	CLIA
PCO_2	± 5 mmHg or 8% (greater)	CLIA
pH	± 0.04	CLIA
PO_2	± 3 SD	CLIA
Potassium (K)	± 0.5 mmol/L	CLIA
Protein, Total (TP)	± 10%	CLIA
Sodium (NA)	± 4 mmol/L	CLIA
Triglycerides (TRIG)	± 25% ± 15%	CLIA NCEP
Urea (UREA)	± 2 mg/dL or 9% (greater)	CLIA
Uric Acid (UA)	± 17%	CLIA

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www.sundiagnostics.us

TAE Limits Table rev20130725

Annexure 3

Medical Decision Points
(Sample from Westgard site)

Medical Decision Levels

These tables of medical decision levels provide possible critical decision levels - where you can assess performance (CV, bias) and determine the Sigma-metrics and appropriate QC procedures.

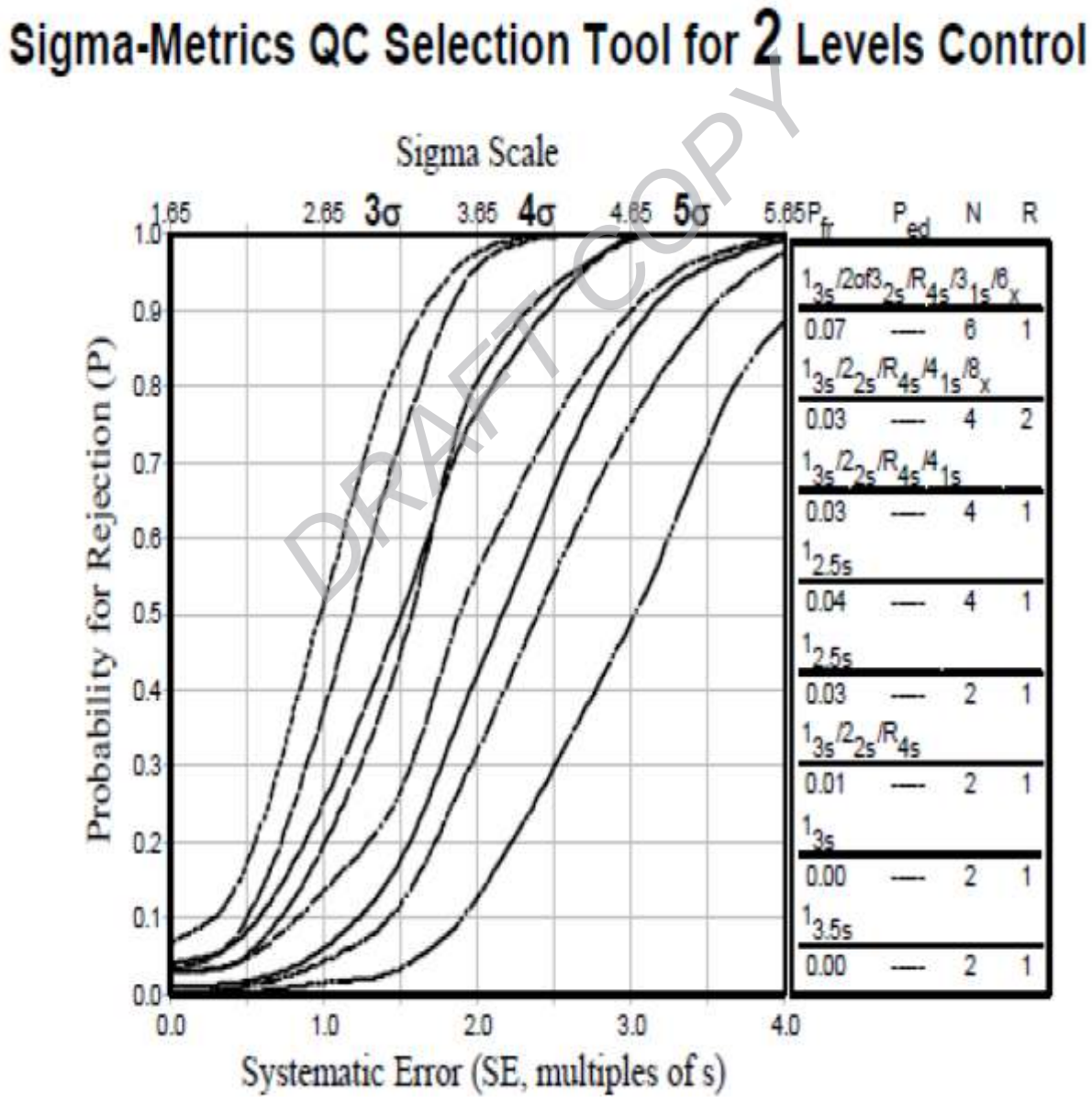
Clinical Decision levels for Electrolytes, Metabolites, Proteins and Enzymes, Hormones, Hematology related tests and Drugs are available. Electrolytes are shown as an example here

Test	Units	Reference Interval	Decision Levels				
ELECTROLYTES			1	2	3	4	5
Calcium	mg/dL	9.0-10.6	7.0	11.0	13.5		
Chloride	mmol/L	98-109	90	112			
CO2 Content	mmol/L	23-30	6.0	20	33		
Magnesium	mEq/L	1.2-2.4	1.2	2.0	5.0		
	mmol/L	0.6-1.2	0.6	1.0	2.5		
Phosphorus	mg/dL	2.5-5.0	1.5	2.5	5.0		
Potassium	mmol/L	3.7-5.1	3.0	5.8	7.5		
Sodium	mmol/L	138-146	115	135	150		

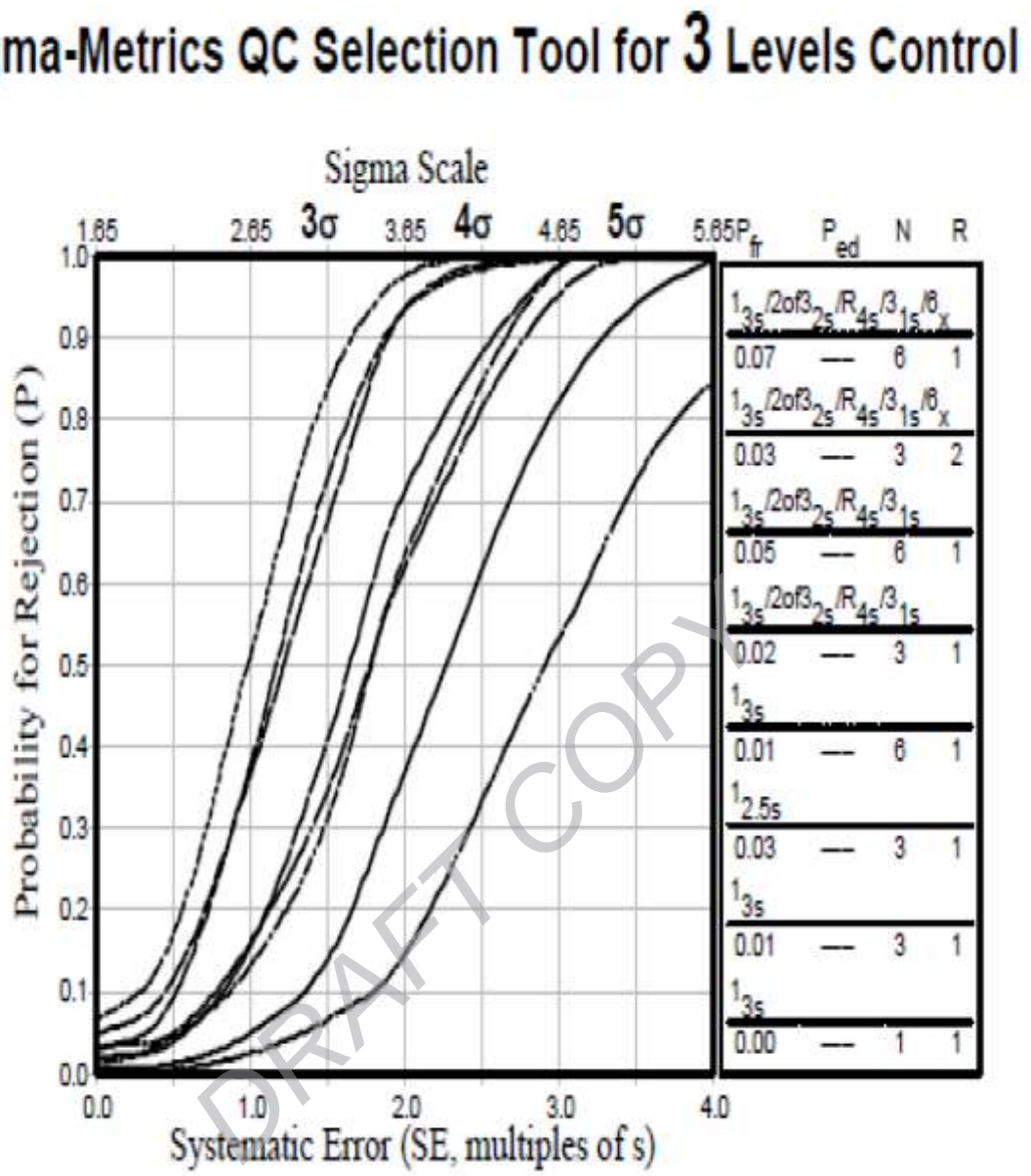
Annexure 4

Sigma-Metrics QC Selection Tools for 2 & 3 Levels Control

A. Sigma-Metrics QC Selection Tool for 2 Levels Control



B. Sigma-Metrics QC Selection Tool for 3 Levels Control



Annexure 5

Frequency and Scope of Testing: Commonly used EQAS Schemes

Frequency and Scope of Testing: Commonly used EQA Schemes in India

Name of the EQAS	Scope	Frequency	Link
CMC Biochemistry EQAS	• Chemistry program I (QCH I) • Chemistry program II (QCH II) • Thyroid Hormones & Cortisol (QT&C) • HbA1c (not suitable for Nycocard method) (QGHB) • Reproductive Hormones (QRPH) • Biochemical Markers for Down's Screening (QDS) • Urine Chemistry (QUC)	*External Quality Assurance Scheme [EQAS] begins in January. *Twelve lyophilized human sera / whole blood samples in batches of four, once every four months	http://home.cmcvellore.ac.in/clinqc/aboutRegistration.aspx
AIIMS EQAS	CBC, Reticulocytes, DLC and Peripheral Smear	One sample once in 3 months	
Histopathology EQA Program by Department of Pathology, All India Institute of Medical Sciences, New Delhi	Two types One for diagnosis/efficacy of reporting by circulating sets of slides amongst Pathologists and collating their diagnoses. Second, for testing the working of the laboratory by testing that labs processing, staining and Quality assurance protocols.	2 or 3 cycles every year.	http://www.pathoindia.com/
Randox EQAS (Called RIAQS)	RIQAS covers 360 parameters across 32 flexible multi-parameter programs. Which are available at its site	Frequency depends upon on the type of the program, some program require samples in every 2 weeks, 2 x 6 monthly cycles and some program require samples every month, 1 x 12 month cycle.	http://www.randox.com/wp-content/uploads/downloads/2016/03/LT033-RIQAS-Explained-FEB16.compressed-5.pdf
Bio-Rad	• Blood Gas Program (12-month cycle) • Blood Typing Program (3 samples tested every 4 months) • Cardiac Markers Program (12-month cycle) • Clinical Chemistry (Monthly) Program (12-month cycle) • Coagulation Program (12-month cycle) • Ethanol/Ammonia Program (12-month cycle)	Biorad follows monthly cycle for EQAS program	http://www.bio-rad.com/en-in/category/external-quality-assurance-services-eqas

Bio-Rad	• Hematology Program (12-month cycle consisting of 4 separate shipments) • Hemoglobin Program (12-month cycle) • HIV/Hepatitis Program • Immunoassay (Monthly) Program • Lipids Program (12-month cycle) • Serum Proteins Program • Syphilis Program • Therapeutic Drug Monitoring Program • ToRCH/EBV/MuMZ Program • Urinalysis Program • Urine Chemistry Program	Biorad follows monthly cycle for EQAS program	http://www.bio-rad.com/en-in/category/external-quality-assurance-services-eqas
CMC Hemostasis EQAS	**Program A:** • Prothrombin time (PT)/INR • Activated thromboplastin time (APTT) • Fibrinogen • Thrombin time (TT) **Program B** • Factor VIII Assay • Factor IX Assay • Von Willebrand factor study • (RICOF & VWF: Ag)	2 Samples Quarterly	https://www.cmceqas.org/registration.php
CMC Transfusion Medicine EQAS:	**Program A (For Laboratories)** • Blood Grouping and Typing **Program B (For Laboratories)** • Blood Grouping and Typing • Direct Coombs and Indirect Coombs test **Program C (For Blood Bank)** • Blood Grouping and Typing • Direct Coombs and Indirect Coombs test • Compatibility test **Program D(For Blood Bank)** • Blood Grouping and Typing • Direct Coombs and Indirect Coombs test • Compatibility test • Antibody Screening • Antibody Identification	One sample once in 3 months	https://www.cmceqas.org/registration.php
Blood Bank External Quality Assessment Scheme (BEQAS)	• HBsAg • Anti-HIV • Anti-HCV • NAT (HBV / HCV / HIV-1,HIV- 2,HIV-O,HIV-M) • VDRL	The frequencies of distribution of samples are 3 cycles per year. (First cycle-January, Second cycle-July, Third cycle-November)	http://nabh.co/Images/pdf/EQAS-ApplicationForm.pdf

	• Malarial Parasite • Hemoglobin • Blood Group • Cross-match • Antibody Screening and Identification		
RML Quality Assurance Program (RML-QAP)	Clinical Biochemistry Immunology Hematology Histopathology & Cytopathology Microbiology & Serology	Six samples in a year (February, April, June, August, October, December) Four samples in a year (March, June, September, December)	http://www.rmlpathology.com/quality-assurance-program#
Tata Memorial Hospital Department Of Cytopathology EQAS - Diagnostic Cytopathology	A set of 5 cytology slides belonging to 5 cases (2 gynaec, 2 non gynaec and 1 FNAC)	Twice in a year	https://tmc.gov.in/newsnevents/Cytology/Cytology%20update/Eqas2012.htm
Indian Academy of Cytologists External Quality Assurance Programme	A set of slides [gynecological (cervical Pap smears) and non-gynecological, FNAC and exfoliative cytology (fluid) smears) are dispatched to the first laboratory in four groups for onward circulation.		http://www.cytoindia.com/Aboutcytoind/presidents.htm
ILQA Bangalore, Anand Diagnostic Lab	• ACP (Monthly) • Biochemistry (17 parameters) –Monthly • Extended Serology (20 parameters) – Half yearly, except Anti HBc which is quarterly • Hematology (16 parameters) – Monthly, but available only for Bangalore local labs • Serology (18 parameters) – Quarterly • Special Chemistry (18 parameters)- Monthly		http://www.ilqabangalore.com/PlanDetails.aspx
Anand Lab Bangalore: Histopathology EQA	**Two groups; A & B** Each group will participate in 3 cycles. **Group A** receives its slides in January, May, and September and should send in their reply within one month. **Group B** will receive its slides in March, July, November and should send in their reply within one month The quality assessment program shall focus on two aspects **PART A:** on pre-analytical aspects beginning from tissue processing, sectioning to staining. **PART B :** on analytical aspects (interpretation of slides).		http://www.ilqabangalore.com/histo/Home.aspx
Immunohistochemistry ILQA program Conducted by QcMark	Two modules are being offered: General module for assorted markers and Breast module for ER, PR and Her-2 testing.	Four markers are offered in each of the three runs in a cycle (year), so covering total 12 markers in a year for general module. The breast module repeats ER, PR and Her in every run (each marker gets tested thrice in a year).	www.QcMark.org

IAMM EQAS	This scheme involves distribution of for quality control packages to be sent during the months of January, April, July and October of every year a) **Staining:** Gram staining/AFB staining/Leishman staining for Malarial parasites. b) **Culture:** General bacteriology ID (Manual/Automated) up to species level and antimicrobial susceptibility (Kirby Bauer/MIC method). c) **Serology:** Antibodies to HIV 1&2 Antibodies to HCV HBs Ag test Widal test CRP RA Factor ASO RPR		http://www.ilqabangalore.com/
The Society for Indian Human and Animal Mycologists (SIHAM) through PGIMER, Chandigarh	Medical Mycology		http://www.siham.in/Media/eqas_in_medical_mycology.pdf
IATP External Quality Assurance in Parasitic Diagnosis through JIPMER, Puducherry	It assesses three major aspects of parasitic diagnosis namely 1) Microscopy 2) Serology 3) Molecular biology.		http://iatp.in/
STI	Syphilis testing by RPR / VDRL / TPHA Gonorrhea Gram Staining/ Antibiotic Susceptibility	Once a year	
NARI	CD4 (Flow Cytometry) – 2 samples thrice in year HIV:8 samples twice yr	Cd4 (Flow Cytometry) – 2 samples thrice in year HIV: 8 samples twice in a year	http://www.nari-icmr.res.in/
EQAS under RNTCP	EQA of the NRLs will be conducted by WHO Supra-National Reference Laboratories. Proficiency testing of DST by the Culture and DST laboratories is conducted at the time of accreditation by the respective designated NRL. The Culture and DST laboratories should send a list of all cultures to NRLs, who would randomly select ten cultures for proficiency testing. These cultures would be then sent to NRLs by 37 Culture and DST laboratories and the result of NRLs will be communicated to the laboratories with corrective actions, if required. In addition, NRLs will send a set of 20 cultures to the laboratories at the time of accreditation and annually thereafter, and the results will be compared and suggestions for improvement would be provided, if required.		

UK NEQAS	NEQAS now comprises a network of 390 schemes operating from 26 centers based at major hospitals, research institutions and universities throughout the UK. The services cover qualitative and interpretative investigations in reproductive science, cellular pathology, clinical chemistry, genetics, hematology, immunology and microbiology.	http://www.ukneqas.org.uk/documents/UKNEQASCompendiumfinal%283%29.pdf
Royal College of Pathologists of Australasia Quality Assurance Programs	Chemical Pathology Group/Program Hematology and Transfusion Group/Program : Hematology & Transfusion Infectious Diseases and Immunology Group/Program: Immunology, Microbiology, Serology, Biosecurity & Synovial Fluid Cellular and Tissue Pathology Group/Program: Anatomical Pathology, Cytopathology Program,	http://www.rcpaqap.com.au http://www.rcpaqap.com.au/wpcontent/uploads/2016/02/2016_Product_Catalogue.pdf

Annexure 6

Corrective Action Formats for IQC & EQA

IQC Corrective Action	
Date	
Analyte	
Sigma	
QC Rules for Analyte	
QC Lot no & Expiry	
QC Level:	
Rule/Rules Violated	
Check Storage/Expiry of	
Reagent	
Calibrator	
QC	
Check Environment	
Temperature	
Humidity	
Check Operator	
Troubleshooting and Corrective actions	
If problem persists, top testing, call service personnel	
Comments	
Signature of Technician	

B. Corrective Action formats for EQA (PT Failure Checklist)

Proficiency Testing Failure Checklist

Survey Name: _____ Clinical Specialty: _____

Specimens: _____ Date: _____

Problem Description: _____

_____

_____

Assessment Review

PT Report Reviewed for Clerical Errors:			
Evaluation results match your copy of submitted results	Yes	No	N/A
Wrong Data Entered	Yes	No	N/A
Wrong Units Reported	Yes	No	N/A
Incorrect instrument or methodology indicated	Yes	No	N/A
Sample Handling:			
Unexpected delays in receiving survey	Yes	No	N/A
Kit contents correct and in acceptable condition	Yes	No	N/A
Testing performed within suggested instructional time guidelines	Yes	No	N/A
Specimens stored at correct temperature between receipt and analysis	Yes	No	N/A
Specimen analyzed at correct temperature	Yes	No	N/A
Sample mixed properly before testing	Yes	No	N/A
Sample diluted properly	Yes	No	N/A
Special Handling instructions were followed	Yes	No	N/A
Testing Procedure:			
Testing Personnel competent to perform analysis	Yes	No	N/A
Manufacturer's package insert available and followed	Yes	No	N/A
Testing procedure properly followed	Yes	No	N/A
Kit components replaced from other kits	Yes	No	N/A
Sample mix-up	Yes	No	N/A
Samples demonstrate a matrix effec	Yes	No	N/A
Instrument recently calibrated or due for calibration	Yes	No	N/A
Instrument maintenance up-to-date	Yes	No	N/A
New lot number of reagents or calibrators used	Yes	No	N/A
Reagents within expiration date	Yes	No	N/A
Results reported within linearity	Yes	No	N/A
QC within established range	Yes	No	N/A
QC demonstrates an even distribution around the mean	Yes	No	N/A
QC results show a shift, trend, or bias	Yes	No	N/A
Manufacturer consulted	Yes	No	N/A
Sample Results:			
A single sample fails on several analytes	Yes	No	N/A
All samples failed for the analyte	Yes	No	N/A
Previous survey results for the analyte demonstrate a problem emerging	Yes	No	N/A
PT material reassayed	Yes	No	N/A

Proficiency Testing Failure Checklist

Survey Name: _____ Clinical Specialty: _____

Specimens: _____ Date: _____

Investigation: _____

_____

_____

_____

_____

_____

Conclusion: _____

_____

Corrective Action Taken: _____

_____

_____

Laboratory Director Review _____

_____

Annexure 7

Evaluation Summary Report

Purpose: Verification of Manufacturer's Claims / Change Controls

Description of Equipment / Process:

Equipment/Process:

Serial Number/ Equipment ID: Reference

Serial Number/ Equipment ID: Test

Date:

FDA Approval Status: Approved / not approved

Procedure:
Ref to Lab QSP: Method Evaluation…..

Results:
All raw data reports and statistical analysis details can be found in the file numbers _____

1. **Precision – refer to file number** _____

   Analyte: _____

MDP	Expected Results		Observed Results		Acceptability
	Manufacturer's Precision Claim	33% of CLIA	**Between Day**		
			Normal Control / Sample CV%	Abn Control / Sample CV%	

MDP	Expected Results		Observed Results		Acceptability
	Manufacturer's Precision Claim	25% of CLIA	**Within Run**		
			Normal Control / Sample CV%	Abn Control / Sample CV%	

2. **Accuracy: refer to file number** _____

Analyte : _____

Total Allowable Error with Source: _____

Results

a) r value _____

b) Slope _____

c) Intercept _____

d) Graph interpretation of Difference and % Difference _____

MDP	Y'	% Bias	%TE	Sigma	Acceptability

3. **Linearity: refer to file number** _____

a) Total Allowable Error and Source _____

b) % of Allowable Error used for calculations _____

c) Graphical Interpretation of Linearity _____

d) Linearity _____

e) AMR _____

f) CRR _____

Assigned Value at Dilution	Mean	Y'	% Diff	% Limit	Acceptability

Analytical Measurement Range (AMR) and Clinical Reportable Range (CRR)

Analyte	Mfg's AMR	Low Value Verified	High Value Verified	Linearity	Validated AMR	Dilutions	CRR

e) Sensitivity and Specificity:

Summary of Manufacturer's Claims for Sensitivity and Specificity		
Analyte	Specificity (Interfering Substances)	Sensitivity
	Icterus – Hemolysis – Lipemia – Drugs –	

5. Reference ranges: refer to file number…

Analyte	Adult Reference Ranges	Reference Range Cited	% Verified (Expected ⩾90%)

Acceptability of Method

1. Manufacturer's claims for linearity, precision and accuracy have been verified
2. The Sigma-metric is _____
3. Biological Reference Interval: Verified/Established/Calculated by Transference

Method Approval

Approved / Not Approved

If not approved, provide recommendations/corrective actions below.

Laboratory Director: _____ Date: _____

Prepared by: _____ Date: _____

WORKSHEETS

Annexure 8

Exercise 1: Find the Mean, Median and Mode

	Mean	Mode	Median
A. 2,2,2,2,42,2,2,2,2,2,2			
B. 9, 2,3,4,11,5,8,6,7,5			
C. 6,6,6,6,6,6,6,6,6,6,6			

Exercise 2.68-95-99 rule for Gaussian

Data set A: Mean 90, SD 3.2

Assign the graph with +_3SD numbers & Plot the data on the graph: Is it Gaussian?

* 93, 84,90,93,88,86,88,95,92,94,88,90,89,87,91,90,94,88,97,90,91,95,90,85,91,94,89.91,85,89

Data Set B: Mean: 52, SD 24

Assign the graph with +_3SD numbers & Plot the data on the graph: Is it Gaussian?

45,48,41,49,102,44,43,141,44,46,43,43,45,49,41,42,40,43,48,43

3. Calculate Mean and SD and Range

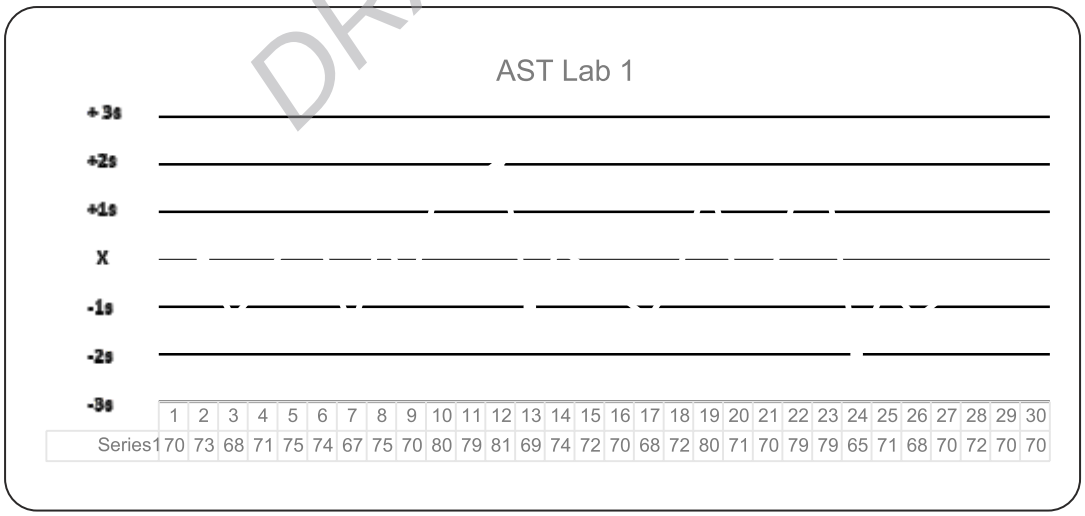
- 5.1, 5.3, 4.9, 5.1, 5.4, 5.1, 5.6, 5.4
- 2.13, 2.09, 2.10, 2.11, 2.15
- 36.83, 35.79, 37.01, 35.72, 36.29, 36.33, 36.54, 36.48, 36.91, 35.87

Exercise 4. LJ Plotting with two levels of QC

Plot the LJ with given values:

Following are the data points for Level 1 QC of AST for the month of September 2016. Please define mean, SD (3SD), range and plot the values on the graph.

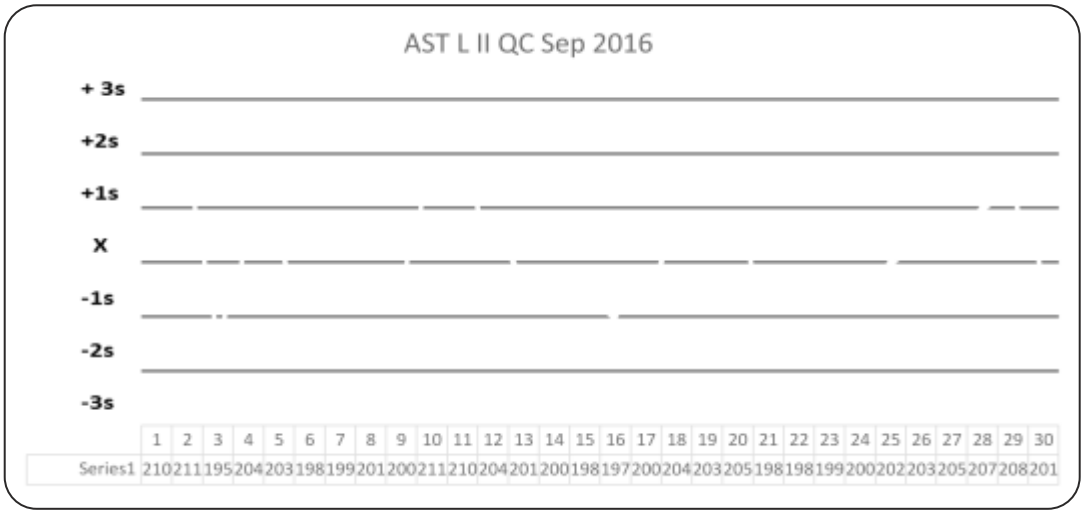
Data Set A:



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Series1	70	73	68	71	75	74	67	75	70	80	79	81	69	74	72	70	68	72	80	71	70	79	79	65	71	68	70	72	70	70

Data Set B:
Following are the data points for Level II QC of AST for the month

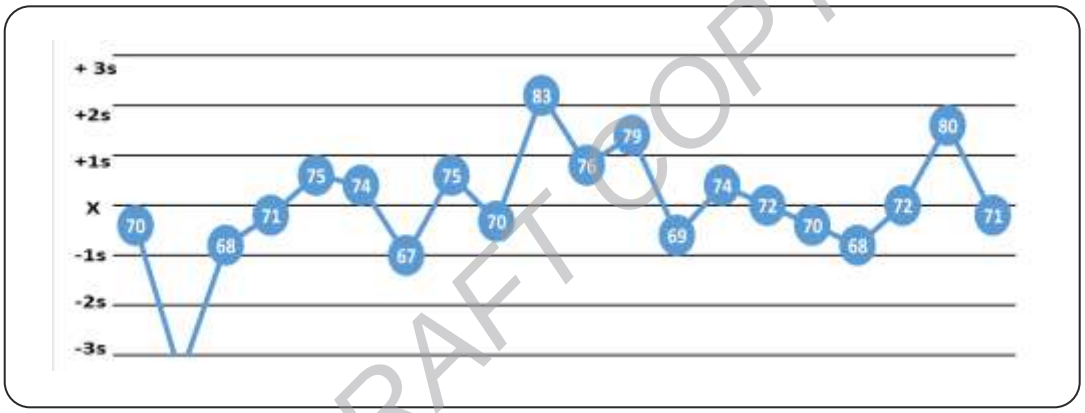
Data Set B:
Following are the data points for Level II QC of AST for the month of September 2016. Please define mean, SD (3SD), range and plot the values on the graph.



Exercise 5 Identify the rule/rules violated

Graph A:

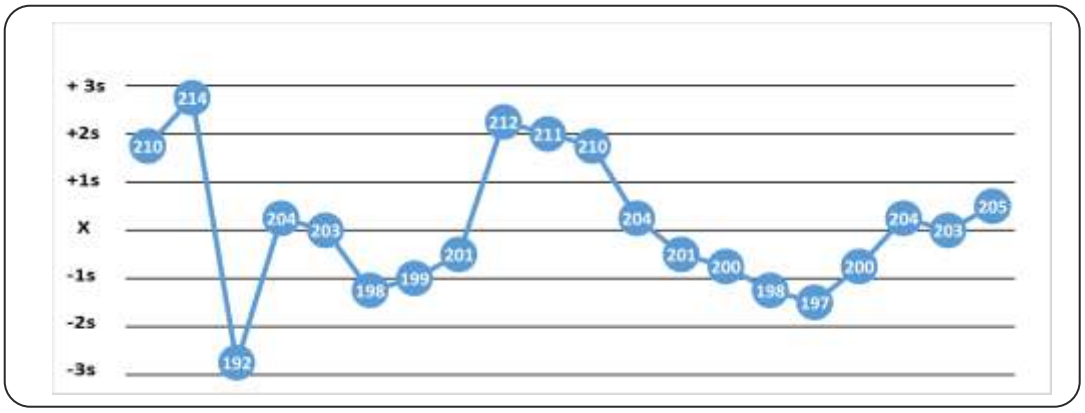
Lab XYZ, October 2015, AST Level 1 QC



Rule/Rules Violated: _____

Graph B:

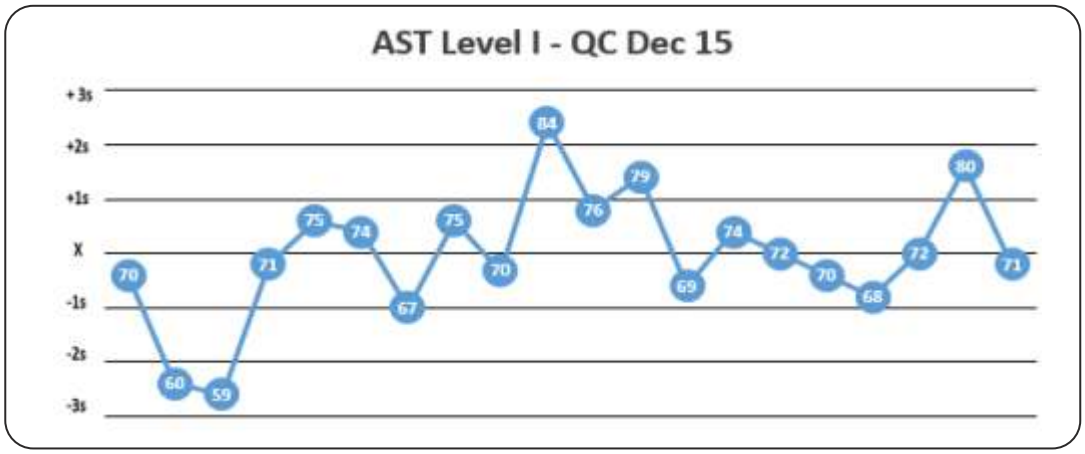
Lab XYZ, Nov 2015, AST Level II QC

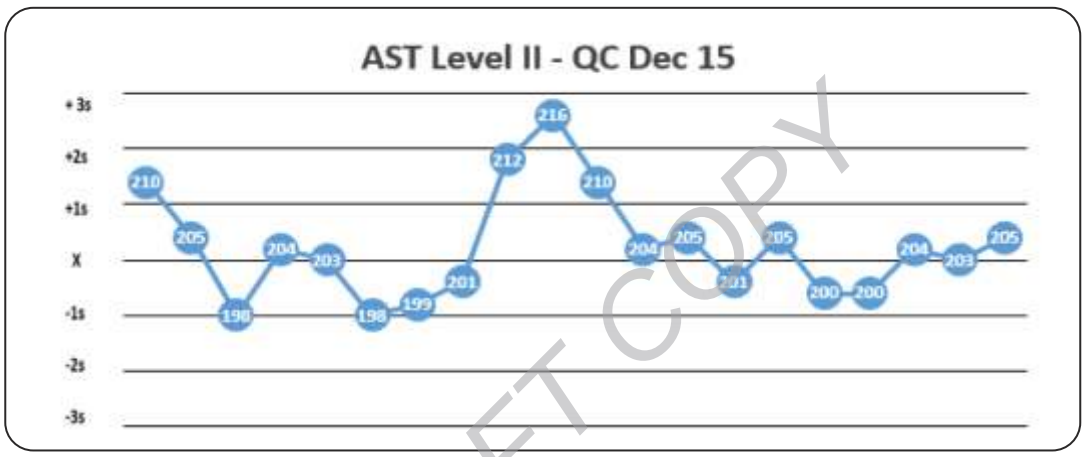


Rule/Rules Violated: _____

Graph 3

Data of L1 & L2 of AST for the month of December 2015.

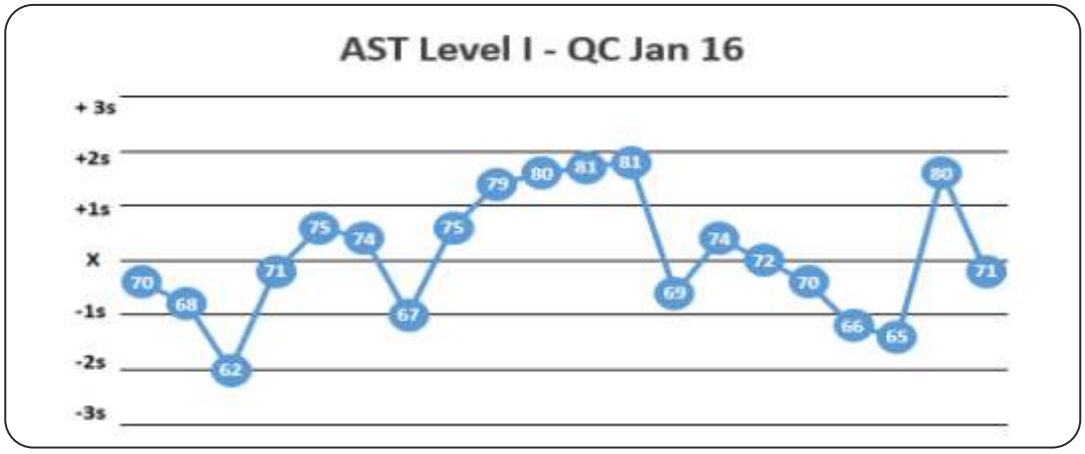


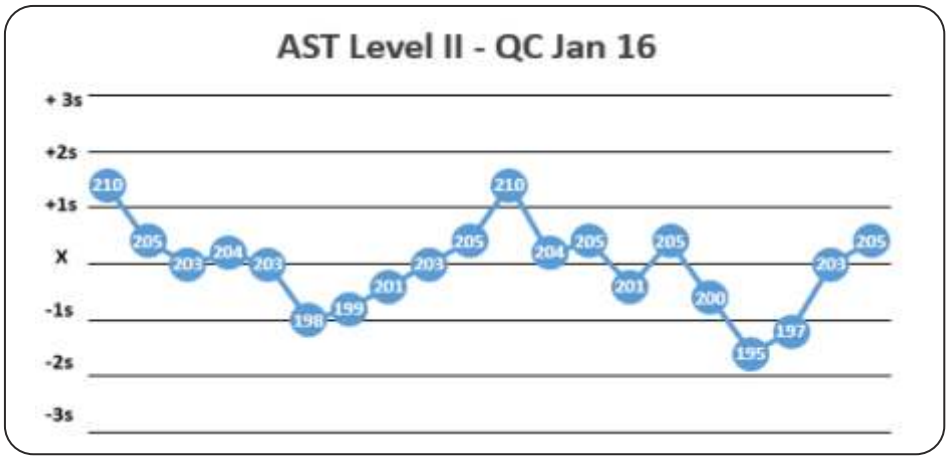


Rule/Rules Violated: _____

Graph 4

Given below are the data points for AST Level I & II for the month of January 2016.





Exercise 6. What errors can be detected on LJ?

Data Set A: 70 68 58 71 75 90 64 75 79 78 80

87 55 74 72 77 66 62 80 71

Plot the LJ with assigned mean and SD and calculate the observed mean and SD for the month of Feb 2016. What kind of error are you seeing and what the possible reasons are for this. What actions will you take to prevent this in future.

87

82

77

72

67

62

57

Assigned Mean: _____ Assigned SD: _____

Observed Mean: _____ Observed SD: _____

Error: _____

Possible reasons:

_____

_____

Corrective Actions:

_____

_____

Preventive Actions:

_____

_____

Data Set B:

73	**72**	**73**	**72**	**70**	**65**	**64**	**65**	**65**	**63**	**63**	**62**	**60**
	63	**61**	**60**	**63**	**62**	**64**	**65**					

Plot the LJ with assigned mean and SD and calculate the observed mean and SD for the month of Feb 2016. What kind of error are you seeing and what the possible reasons are for this. What actions will you take to prevent this in future.

87 _____

82 _____

77 _____

72 _____

67 _____

62 _____

57 _____

Assigned Mean: _____ Assigned SD: _____

Observed Mean: _____ Observed SD: _____

Error: _____

Possible reasons:

_____

_____

Corrective Actions:

_____

_____

Preventive Actions:

_____

_____

Exercise 7: Calculating CV percentage

The following are the data points for Level I & II for AST for the month of January 2015 for Lab XYZ.

Data Set A:

70	68	62	71	75	74	67	75	79	80	81	81	69
	74	72	70	66	65	80	71	70	79	79	65	71
	68	70	72	70	70							

Mean _____

SD _____

CV%: _____

Data Set B:

210	205	203	204	203	198	199	201	203	205	210	204	205
	201	205	200	195	197	203	205	198	198	199	200	202
	203	205	207	208	201							

Mean _____

SD _____

CV%: _____

Exercise 8: New Lot QC

Scenario A: You have a new lot of QC no 12345. For AST Level II, the manufacturer's mean is 220 IU/L and range is 190-250 IU/L. You have done parallel testing and got these values. Plot your lab's chart for Lot No. 12345 for AST, before you would assign a new range and new mean.

210	**205**	**203**	**204**	**203**	**198**	**199**	**201**	**203**	**205**	**210**	**204**	**205**
	201	**205**	**200**	**195**	**197**	**203**	**205**	**198**	**198**	**199**	**200**	**202**
	203	**205**	**207**	**208**	**201**							

What will be the Lab assigned mean and range?

Manufacturer's mean: 220 IU/L Lab Mean _____

Manufacturer's Range: 19-250 IU/L Lab Range _____

+3s _____

+2s _____

+1s _____

X _____

-1s _____

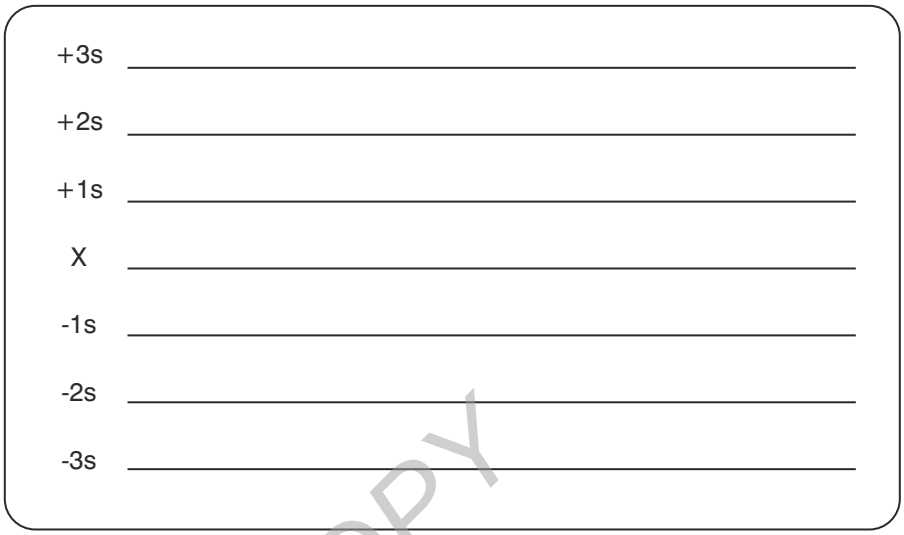
-2s _____

-3s _____

Scenario B:

Scenario A: You have a new lot of QC no 12345. For AST Level II, the manufacturer's mean is 220 IU/L and range is 190-250 IU/L. You could not do the parallel testing in full because QC was supplied late. You have accumulated 8 data points over 4 days as shown below. CV% for AST in the running/current lot 12344 is 4%. Plot your lab's chart for Lot No. 12345 for AST, before you would assign a new lab range and lab mean.

No. of run	Values
1	202
2	205
3	210
4	204
5	203
6	201
7	199
8	194
Mean	
SD	
CV %	



What will be the Lab assigned mean and range?

Manufacturer's mean: 220 IU/L Lab Mean _____

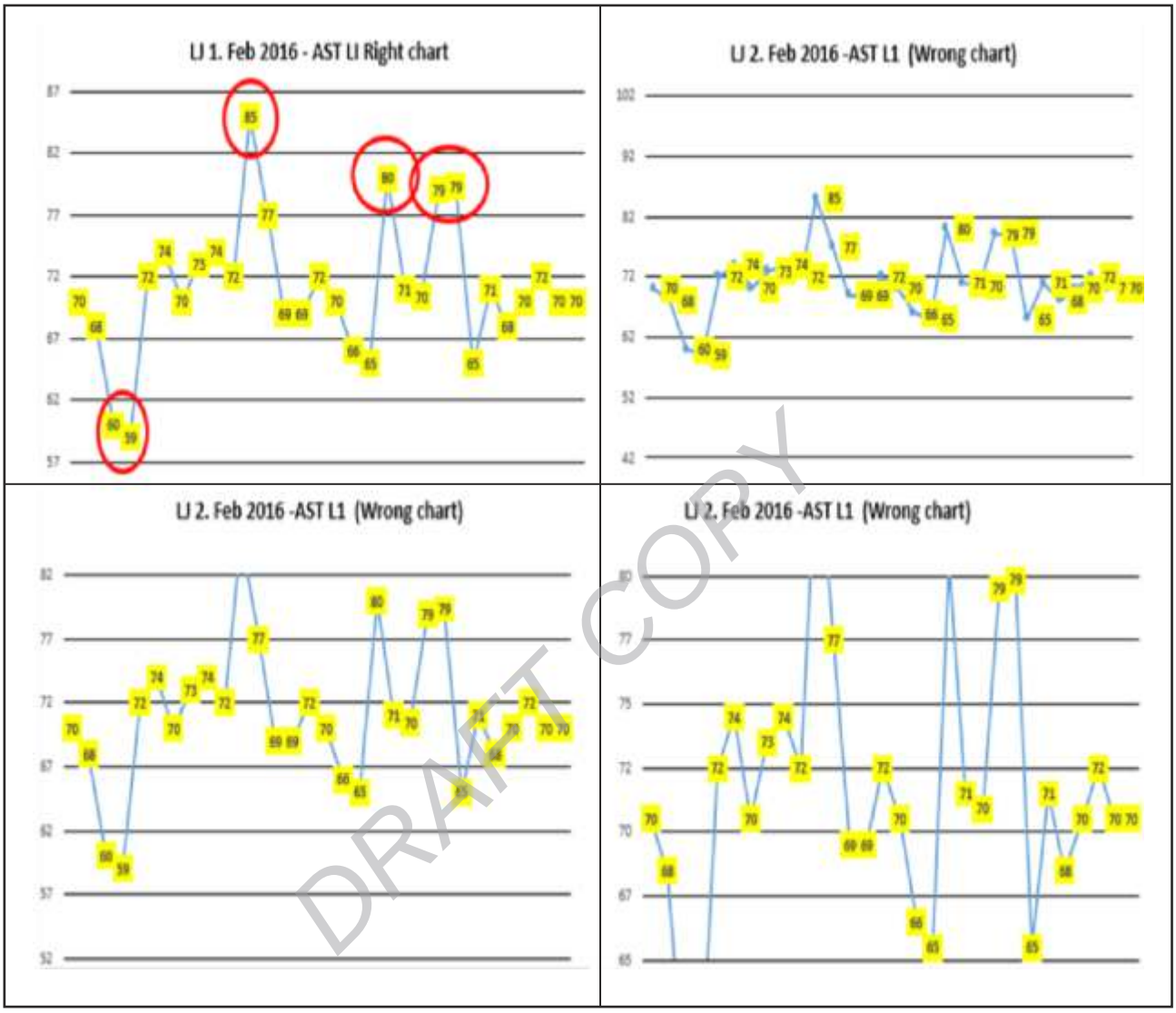
Manufacturer's Range: 19-250 IU/L Lab Range _____

Exercise 9: Right and Wrong LJ Chart

For the data given below for Level 1 AST control, four charts have been plotted. Out of them one is correct and others are wrong (marked accordingly). Identify the problem in the charts and the consequences of using wrong charts. Specific inputs are to be given for the circled data points.

Exercise 9: Right and Wrong LJ Chart

For the data given below for Level 1 AST control, four charts have been plotted. Out of them one is correct and others are wrong (marked accordingly). Identify the problem in the charts and the consequences of using wrong charts. Specific inputs are to be given for the circled data points.



Exercise 10: Bias, Absolute Bias, % Bias

For the data given below, calculate Bias, Absolute Bias,, % Bias.

Data of Glucose & AST for the month of November 2015 is

	Glucose	AST
Lab Mean	**95**	**203**
Peer group Mean	**90**	**197**
Bias		
Absolute Bias		
% Bias		

Exercise 11: Total Error (TE), %TE

For the data given below, calculate the % CV, Total Error (TE), %TE.

Data of Glucose & AST for the month of November 2015 is

	Glucose	AST
Lab Mean	95	203
Peer group Mean	90	197
SD	4	6
% CV		
Absolute Bias		
% Bias		
TE		
%TE		

Exercise 12: Total Allowable error and judging acceptability of the analyte performance

Find the TE_A using CLIA proficiency limits from annexure and compare with the total error in the above cases and judge acceptability of the analyte performance.

	Glucose	AST
%TE		
% TE_A from CLIA		
Judging Acceptability		

Exercise 13: Sec & Sigma

Using the data from above, calculate the Sec and Sigma.

	Glucose	AST
Lab Mean	95	203
Peer group Mean	90	197
Bias		
Absolute Bias		
% Bias		
SD		
%CV		
TE		
%TE		
% TE_A		
Sigma		
Sec		
Judging Acceptability		

Exercise 14: Rule Selection

Using the data from the exercises 10 to 14, and by using the sigma scale tool (given in annexure), decide the QC rules to be followed for each of the analytes in your lab.

QC rules for Glucose: _____

QC rules for AST: _____

Answer Keys of the worksheets

Exercise 1:

A. Mean: 5.64, Mode: 2, Median: 2

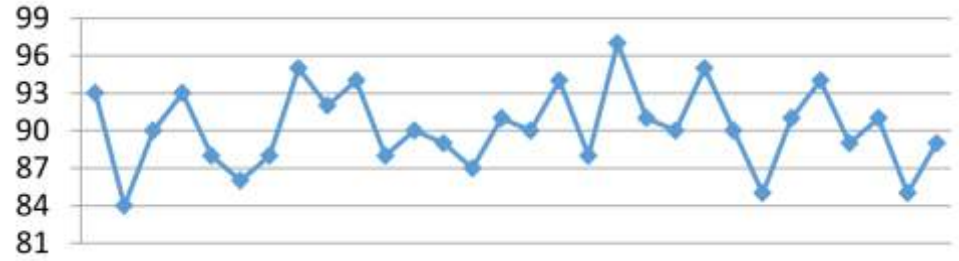
B. Mean: 6, Mode: 5, Median: 5.5

C. Mean: 6, Mode: 6, Median: 6

Exercise 2:

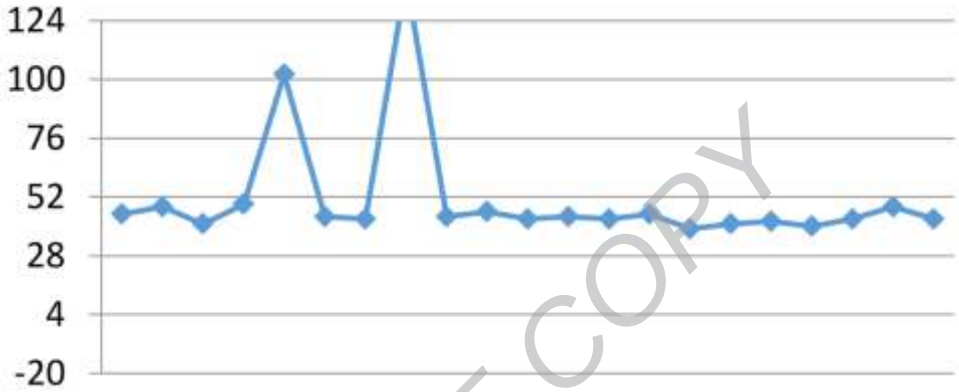
Graph .1

.



YES THIS IS GAUSSIAN

Graph .2



These numbers will not form a Gaussian pattern. A -2SD is a negative number. The mean and median are far apart

Exercise 3

Data Set	A	B	C
Mean	5.24	2.1	36.38
SD	0.23	0.02	0.47
Upper End	5.92	2.19	37.78
Lower End	4.56	2.04	34.97

Exercise 4

	Data Set A	Data Set B:
Mean	72.4	202.5
SD	4.3	4.4
Upper End	85.3	215.7
Lower End	59.5	189.3

Exercise 5:
Graph 1

Data points	Rules
2	1:3S
10	1:2S

Graph 2

Data points	Rules
2	1:2s
3	1:2s
9-11	3:1s
9-16	7 T

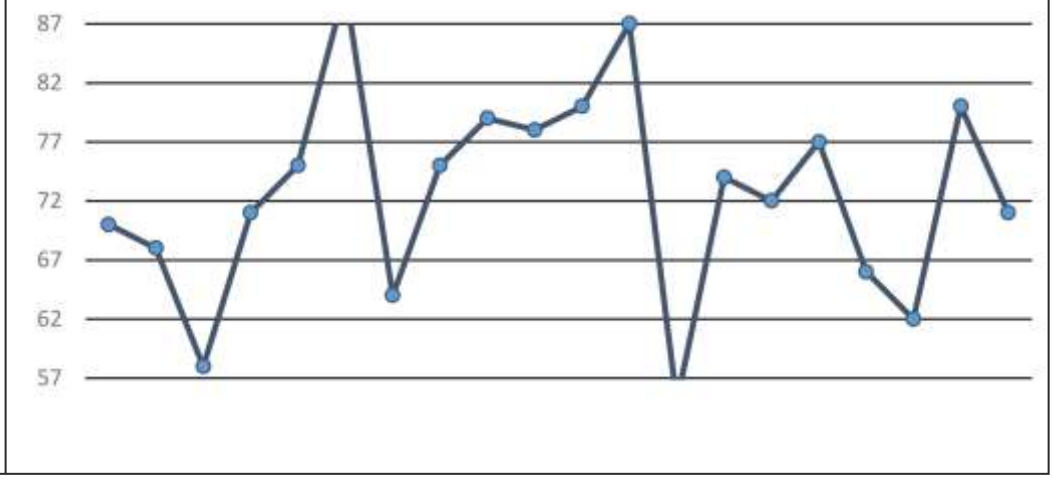
Graph 3

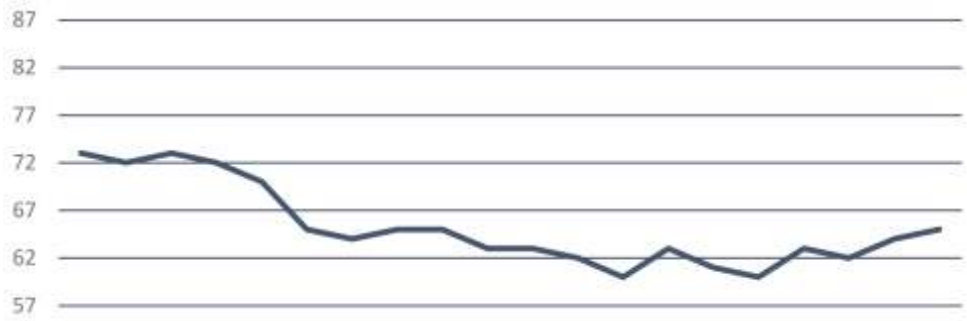
Data points	Rules	
2&3 L1	2:2S	Within material, across run
10 of L1 &2	2:2S	Within run, across material

Graph 4

Data points	Rules	
9 to 13	4:1S	Within material, across 4 runs
17 to 18	4:1S	Across material, across 2 runs

Exercise 6

LJ Data Set A	

LJ Data Set B	

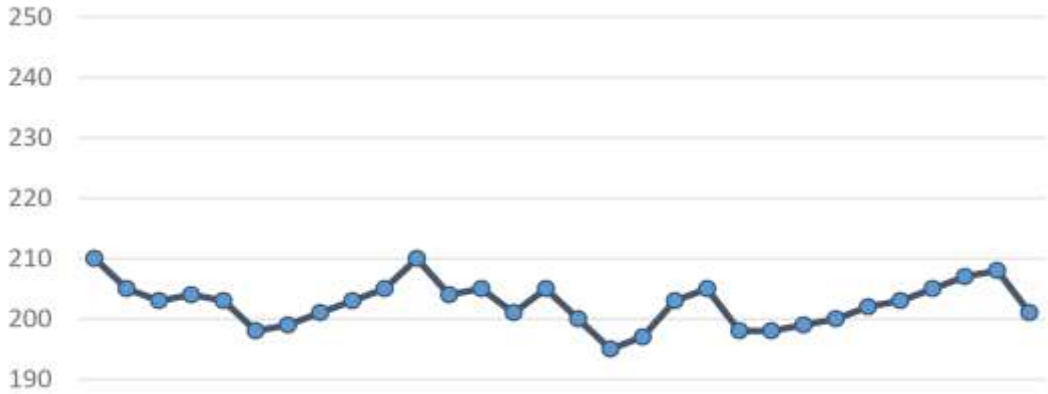
	Data Set A	Data Set B
Assigned Mean	72	72
Assigned SD	5	5
Observed Mean	72.6	**65.25**
Observed SD	8.9	**4.30**
Error	Increasing imprecision, widening SD, Errors in the tails, because of Random Errors	Systematic Error, Shifting Mean. Shifting Accuracy without much change in SD
Possible reasons	All causes of random error	All causes of Systematic error

Exercise 7:

	Data Set A	Data Set B
Mean	72.14	203
SD	5.20	3.7
CV %	7.2	1.8

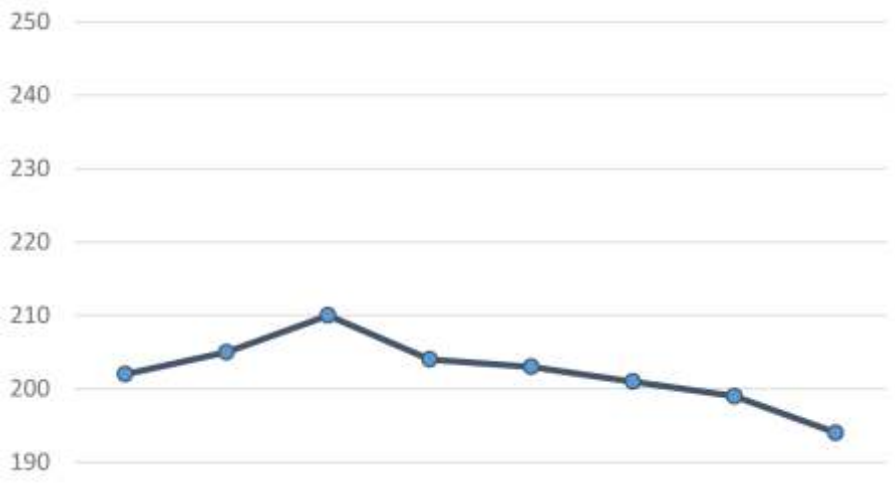
Exercise 8:

Data Set A:



Mean	202.6
Range	191.5-213.7
SD	3.7

Data Set B:



Current Lot CV	4
New Lot Mean	202
New Lot SD	8.1
New Range	178-226

Exercise 9:

Left Upper: Right Plot

Left Lower: Wrong mean

Right Upper: Too large SD

Right Lower: Too low SD

Exercises 10 to 14

	Data Set A	Data Set B:
Lab Mean	95	203
Peer group Mean	90	197
Bias	5	6
Absolute Bias	5	6
% Bias	5.6	3.0
SD	4	6
%CV	4.2	3.0
TE	11.6	15.9
%TE	12.5	7.9
% TEa	10	20
Sigma	1.1	5.7
SEc	-	4.1
Judging Acceptability	Not Acceptable	Good Performance
Rule Selection	Change Method	Single Rule