QC Education

Basic Statistics in Laboratory Quality Control

QC Workbook Series



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How to Use This Workbook

This booklet is part of an educational series offered by Bio-Rad, designed to improve your knowledge of QC statistics and their use. After review of the materials, practice evaluation charts are available beginning on pages 23 and 31.

A Certificate of Completion is available with successful completion of a short test on the materials. Please see page 33 for details and link to the exam.

Bio-Rad proudly supports laboratory professionals that are committed to achieving a higher standard of quality for improved patient test results. Educational materials are continually in development to support the quality journey.

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Introduction

Achieving quality in the medical laboratory requires the use of many tools. These include procedure manuals, maintenance schedules, calibrations, training, quality control and a quality assurance program.

This workbook explains the basic knowledge necesssary to understand a simple but effective statistical process control system. Statistical process control is the use of statistical techniques to verify the reliability of patient results. It is based on statistics calculated from the regular testing of quality control materials.

What will you learn?

- Define and apply the basic elements of quality control
- Define, calculate and apply the following statistical terms: mean, standard deviation, coefficient of variation, coefficient of variation ratio and standard deviation index
- Describe and review the different QC statistical rules
- Identify which statistical rules identify random error and which rules identify systematic error
- Identify and differentiate between trends and shifts
- Identify and differentiate between random error and systematic error
- Construct a Levey-Jennings chart and evaluate graphed data for out of control events
- Assess instruments, reagents, and control products using the coefficient of variation
- Evaluate within lab precision using the coefficient of variation ratio
- Evaluate laboratory trueness using the standard deviation index

The laboratory quality system

A robust quality system permeates all laboratory activities from the point of the test order and patient reception to the analytical bench ending with the report of the patient test result to the appropriate individual.

One of the most important parts of the overall laboratory quality system is the Quality Control Procedure.

The Quality Control Procedure consists of the following:

- Quality control materials
- Statistical process control
- Retrospective review of data

QC results are used to evaluate whether the instrument is operating within pre-defined specifications, inferring that patient test results are reliable.

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What is Quality Control?

One important part of quality control in the medical laboratory is the statistical process used to monitor and evaluate the analytical process that produces patient results. Quality control data are produced by testing quality control materials in the same manner as patient samples.

When a test is performed in the medical laboratory, the outcome of the test is a result. The result may be a patient result or it may be a quality control (QC) result. The result may be quantitative (*i.e.,* a number) or qualitative (*e.g.,* positive or negative) or semi-quantitative (*e.g.,* +1, +2, +3, +4)[1].

QC results are used to evaluate whether the instrument is operating within pre-defined specifications, inferring that patient test results are reliable. Once the test system is evaluated, patient results can then be used to support diagnosis, prognosis, or treatment planning. For example, when a patient's serum is tested for potassium, the test result tells us how much (concentration) potassium is present. This result is then used by the physician to support the determination that the patient has a low, normal or high potassium.

Let's assume the measured value of potassium in a patient's serum is 2.8 mmol/L (a unit of measure, millimoles per liter).[2]

This result is abnormally low and indicates an inappropriate loss of potassium. But how does the person performing the test know that this result is truly reliable? It could be possible that the instrument is out of calibration and the patient's true potassium value is 4.2 mmol/L – a normal result. The question of reliability for most testing can be resolved by regular use of quality control materials and statistical process control.

In this workbook the basic statistics to evaluate test performance are explained.

[1]This workbook will deal only with the quality control of quantitative data.
[2]Potassium can also be measured as milliequivalents per liter (mEQ/L).

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Comparison of Quality Control Results to Specific Statistical Limits

In Table 1, there are two ranges listed. The acceptable range for level 1 is 3.7 – 4.3 mmol/L. The range for level 2 is 6.7 – 7.3 mmol/L[3].

When the daily QC result obtained for the level 1 control is compared to the range calculated for the level 1 control, it becomes apparent that each result lies somewhere within the expected range. This indicates that the analytical process is "in control" at the normal level on that day of testing.

When the daily QC result for the level 2 control (high potassium) is compared to the defined range for the level 2 control, the analytical process is shown to be "in control" for each day of testing except for the last day (Nov 7). On November 1 through November 6, both controls were "in control" and patient values could be reliably reported. However, the laboratory was "out of control" for abnormal high potassiums on November 7 because the value obtained for the QC material (8.0 mmol/L) was outside the acceptable range (6.7 – 7.3 mmol/L).

This means that an error occurred which made the patient results unreliable. The laboratory should not report the test results from any patient samples until the error is resolved and the samples are re-tested.

Perhaps it is now apparent that the range defined for each level of control is fundamental to the quality control procedure. The next section describes how to calculate the basic statistics required to develop an acceptable control range.

Table 1: Example of a QC Result Table With Patient Results.

Test: Potassium	Instrument: Number 1		Unit of Measure: mmol/L
	Level 1	Level 2	Patient Results
Range	3.7-4.3 mmol/L	6.7-7.3 mmol/L	
November 1	4.0	7.0	4.2, 4.0, 3.8, 5.0, 5.8, 4.2
November 2	4.1	7.0	3.8, 4.4, 4.6, 3.9, 4.8, 4.4, 3.9
November 3	4.0	6.9	4.4, 3.9, 3.7, 4.7
November 4	4.2	7.1	4.7, 5.6, 4.2, 3.7, 4.3
November 5	4.1	7.0	4.2, 4.3, 4.1, 4.3
November 6	4.1	7.0	4.6, 4.4, 5.5, 3.8, 3.2
November 7	4.2	8.0	2.8, 4.6, 4.2, 3.2, 3.9, 4.1, 6.0, 4.3

[3]Normal and abnormal are used in the workbook, though for QC materials there could be more levels.

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Calculation and Use of QC Statistics

QC statistics for each test performed in the laboratory are calculated from the QC data collected by regular testing of control products. The data collected is specific for each level and lot number of control. Consequently, the statistics and ranges calculated from this data are also specific for each level and lot number of control and reflect the behavior of the test at specific concentrations. The most fundamental statistics used by the laboratory are the mean [$\bar{x}$] and standard deviation [s].

Calculating a Mean [$\bar{x}$]

The mean (or average) is the laboratory's best estimate of the analyte's true value for a specific level of control.

To calculate a mean for a specific level of control, first, add all the values collected for that control. Then divide the sum of these values by the total number of values. For instance, to calculate the mean for the Level 1 control in Table 1, find the sum of the data {4.0, 4.1, 4.0, 4.2, 4.1, 4.1, 4.2}. The sum [$\sum$] is 28.7 mmol/L. The number of values is 7 (n = 7). Therefore, the mean for the Level 1 potassium control in Table 1 from November 1-7 is 4.1 mmol/L (or 28.7 mmol/L divided by 7).

Formula 1: Calculating the Mean [$\bar{x}$]

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

Where:

$\sum$ = Sum

x_i = Each value in the data set

n = The number of values in the data set

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Calculating a Standard Deviation [s]

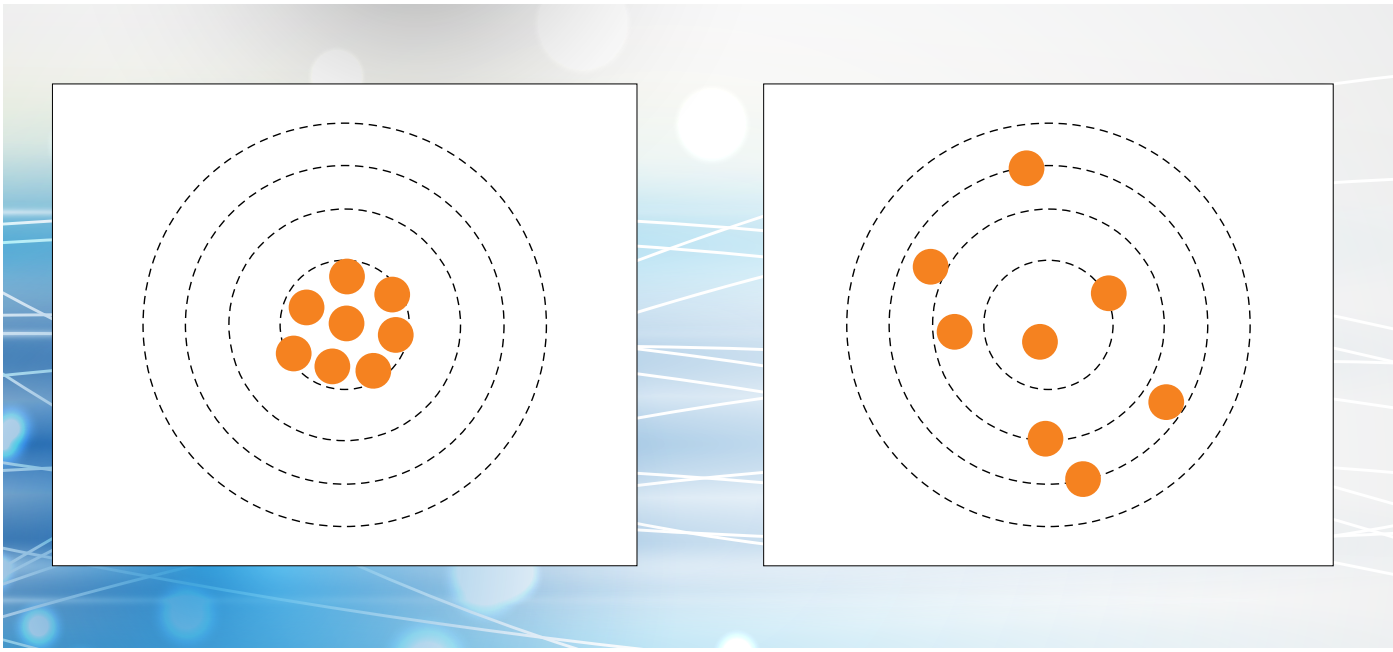
Standard deviation is a statistic that quantifies how close numerical values (i.e., QC values) are in relation to each other. Standard deviation as a word can be abbreviated as SD. In equations and mathematics the sample standard deviation is abbreviated by the Latin letter “s”.

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.

It is desirable to get repeated measurements of the same specimen as close as possible. Good precision is especially needed for tests that are repeated regularly on the same patient to track treatment or disease progress. For example, a diabetic patient in a critical care situation may have glucose levels run every 2 to 4 hours. In this case, it is important for the glucose test to be precise because lack of precision can cause loss of test reliability. If there is a lot of variability in the test performance (high imprecision, high standard deviation), the glucose result at different times may not be reliable.

Figure 1: Example of Good Precision (Low Imprecision)

Figure 2: Example of Poor Precision (High Imprecision)



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Standard deviation may also be used to monitor on-going day-to-day performance. For instance, if during the next week of testing, the standard deviation calculated in the example for the normal potassium control increases from 0.08 to 0.16 mmol/L, this indicates a serious loss of precision. This instability may be due to a malfunction of the analytical process. Investigation of the test system is necessary and the following questions should be asked:

- Has the reagent or reagent lot changed recently?
- Has maintenance been performed routinely and on schedule?
- Does the potassium electrode require cleaning or replacement?
- Are the reagent and sample pipettes operating correctly?
- Is the quality of the water used for washing the system still within specifications?
- Was a new test operator added recently?
- Has a calibration been performed recently?

Although most calculators and spreadsheet programs automatically calculate standard deviation, it is important to understand the underlying mathematics.

Formula 2: Calculating a Standard Deviation [s] for a Set of QC Values

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

Where:

s = Standard deviation

$\bar{x}$ = Mean (average) of the QC values

$\sum(x_i-\bar{x})^2$ = The sum of the squares of differences between individual QC values and the mean

n = The number of values in the data set

$\sum$ = Sum

x_i = Each value in the data set

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To calculate the standard deviation for the normal level of control (level 1) in Table 1, begin by calculating the mean [$\bar{x}$]:

$$\bar{x} = 4.0 + 4.1 + 4.0 + 4.2 + 4.1 + 4.1 + 4.2 \text{ mmol/L} \div 7$$
$$\bar{x} = 28.7 \text{ mmol/L} \div 7$$
$$\bar{x} = 4.1 \text{ mmol/L}$$

Calculate the standard deviation [s] as follows:

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

$$s = \sqrt{\frac{(4 - 4.1)^2 + (4.1 - 4.1)^2 + (4 - 4.1)^2 + (4.2 - 4.1)^2 + (4.1 - 4.1)^2 + (4.1 - 4.1)^2 + (4.2 - 4.1)^2}{6}}$$

$$s = \sqrt{\frac{(-0.1)^2 + (0.0)^2 + (-0.1)^2 + (+0.1)^2 + (0.0)^2 + (0.0)^2 + (+0.1)^2}{6}}$$

$$s = \sqrt{\frac{0.01 + 0.0 + 0.01 + 0.01 + 0.0 + 0.0 + 0.01}{6}}$$

$$s = \sqrt{\frac{0.04}{6}}$$

$$s = 0.082 \text{ OR } 0.1 \text{ (Rounded)}^4$$

The standard deviation for one week of testing of the normal potassium control level is 0.082 mmol/L.[5]
Now that the amount of precision is known, some assumptions can be made about how well this test is performing.

[4]Rounding of the mean and standard deviation to the nearest tenth is allowable because in this example potassium results are generated and reported to the nearest tenth. The standard deviation of 0.08 mmol/L is rounded to 0.1 mmol/L.
[5]This type of standard deviation is called between run standard deviation because the data used to calculate the statistics came from different analytical runs.

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Coefficient of Variation [CV]

The Coefficient of Variation [CV] is the ratio of the standard deviation to the mean and is expressed as a percentage.

The CV allows the laboratory professional to make easier comparisons of the overall precision. Since standard deviation typically increases as the concentration of the analyte increases, the CV can be regarded as a statistical equalizer. If the laboratory professional is comparing precision for two different methods and uses only standard deviation, he or she can be easily misled. For example, a comparison between hexokinase and glucose oxidase (two methods for assaying glucose) is required. The standard deviation for the hexokinase method is 4.8 and it is 4.0 for glucose oxidase. If the comparison only uses standard deviation, it can be incorrectly assumed that the glucose oxidase method is more precise than the hexokinase method. If, however, a CV is calculated, it might show that both methods are equally precise. Assume the mean for the hexokinase method is 120 and the glucose oxidase mean is 100. The CV then, for both methods, is 4%. They are equally precise.

Formula 3: Calculating the Coefficient of Variation [CV]

$$CV = \left(\frac{S}{\bar{X}} \right) 100$$

Where:

S = Standard deviation

$\bar{X}$ = Mean

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The Coefficient of Variation can also be used when comparing instrument performance. Consider the data in Table 2.

In the example shown in Table 2, Instrument #1 and Instrument #2 have similar precision for calcium and glucose, but Instrument #1 demonstrates much better precision than Instrument #2 for phosphorus. Because the precision was calculated from data for the same QC lot number and level of control, the differences in precision are likely due to the instrument or reagent.

Table 2: Imprecision Differences Due to Instrument or Reagent

	Level 1 Chemistry Control (Normal) Lot number 12345	
	Instrument 1 / Reagent 1	Instrument 2 / Reagent 2
Analytes	CV	CV
Calcium	6.1%	5.9%
Glucose	4.4%	4.2%
Phosphorus	5.2%	9.9%

In Table 3, the difference in performance is probably due to the change from reagent #1 to reagent #2. However, it could also be due to lack of regular maintenance or some other cause.

Table 3: Imprecision Differences Due to Reagent or Lack of Regular Maintenance

	Level 1 Chemistry Control (Normal) Lot number 12345	
	Instrument 1 / Reagent 1	Instrument 1 / Reagent 2
Analytes	CV	CV
Calcium	4.2%	6.8%

The data in Table 4 is for three different assay reagent kits for testing ß-hCG. Kits #1, #2 and #3 exhibit similar performance in the normal range (level 2) and at the high end (level 3) of the method curve. However, Kit #3 has a much higher CV at the low end (level 1) of the curve. This lack of precision at the low end of the method curve for ß-hCG provides justification to use either Kit #1 or #2, rather than Kit #3 for testing. Imprecision and inaccuracy are most important at the clinical decision levels. For ß-hCG, the clinical decision levels are at low concentrations (corresponding to the early pregnant state in the female and early testicular cancer in the male) or at moderate concentrations (to diagnose the progression of pregnancy).

Table 4: Imprecision Differences Due to the Use of Different Kits for Testing

	Level 1 Immunoassay (Low) Lot number 12345	Level 2 Immunoassay (Normal) Lot number 12345	Level 3 Immunoassay (High) Lot number 12345
	Test: β-hCG	Test: β-hCG	Test: β-hCG
Kit Number	CV	CV	CV
Kit 1	6.0%	4.5%	12%
Kit 2	5.7%	5.0%	10%
Kit 3	15.0%	4.7%	11%

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The previous examples have shown how CV can be used to compare and evaluate instruments or reagents. So, what is an acceptable CV?

There are several sources which may be referenced to determine expected levels of precision.
These include:

- Precision information provided in the assay reagent insert or instrument manual
- Interlaboratory comparison programs
- Proficiency surveys[6]
- Evaluations of instruments and methods published in professional journals
- Regulatory organizational proficiency limits (*e.g.* CLIA)

[6]When comparing the Coefficient of Variation always be sure to compare normal levels to normal levels, abnormal highs to abnormal highs, abnormal lows to abnormal lows.

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Levey-Jennings Charts and Statistical Rules

Creating a Levey-Jennings Chart

Standard deviation is commonly used for preparing Levey-Jennings (L-J or LJ) charts. The Levey-Jennings chart is used to graph successive (run-to-run or day-to-day) quality control values. A chart is created for each test and level of control. The first step is to calculate decision limits.

These limits are 1 standard deviation (±1s) , 2 standard deviations (±2s) and 3 standard deviations (±3s) from the mean. The mean for the level 1 potassium control in Table 1 is 4.1 mmol/L and the standard deviation is 0.1 mmol/L. Formula 4 provides examples on how ±1s, ±2s and ±3s quality control limits are calculated.

Formula 4: Calculating Quality Control Limits

±1s range: $\bar{x} \pm s$
±2s range: $\bar{x} \pm 2.s$
±3s range: $\bar{x} \pm 3.s$

$\bar{x}$ = mean
s = standard deviation

These ranges are used with the mean to construct the Levey-Jennings chart as shown in Figure 3.

±1s range is 4.0 to 4.2 mmol/L
4.1 – (0.1)(1) = 4.0
4.1 + (0.1)(1) = 4.2

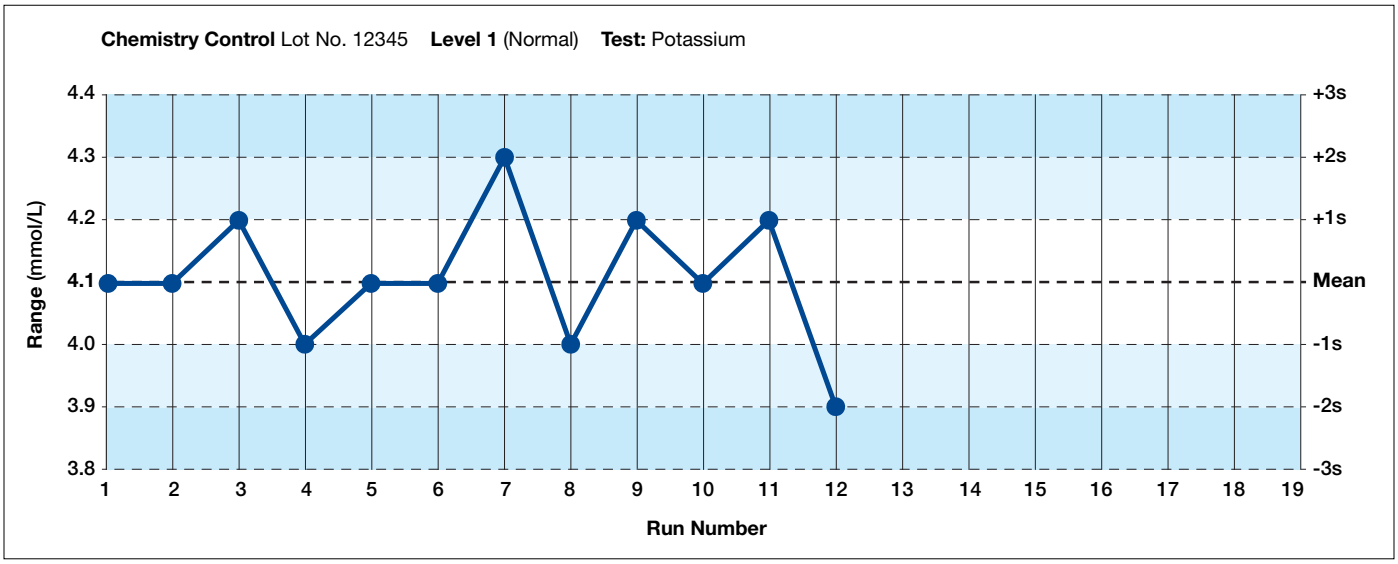
±2s range is 3.9 to 4.3 mmol/L
4.1 – (0.1)(2) = 3.9
4.1 + (0.1)(2) = 4.3

±3s range is 3.8 to 4.4 mmol/L
4.1 – (0.1)(3) = 3.8
4.1 + (0.1)(3) = 4.4

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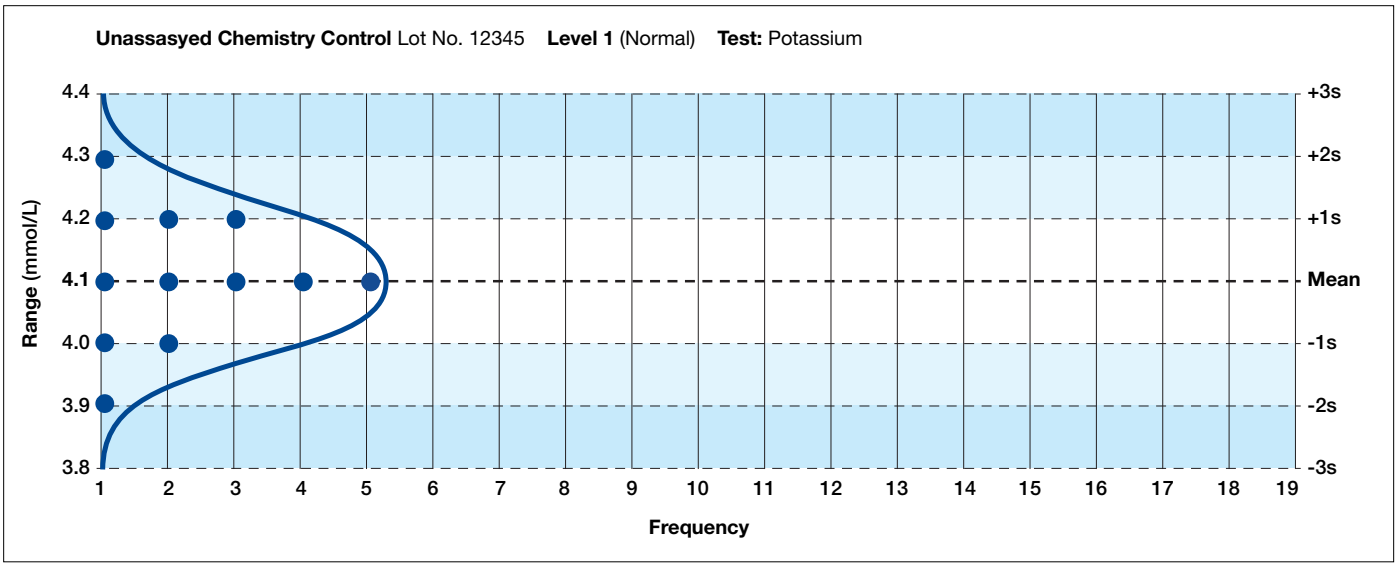
The chart in Figure 3 was introduced by Levey and Jennings in 1950. On the right side of the chart there is an indication of the standard deviations. On the left side of the graph there is an indication of the values. On the left side of the graph the values corresponding to the standard deviations are listed. The bottom of the graph shows the run number, which in other graphs could also be labeled as the time/date.

Figure 3: Levey-Jennings Chart



The Levey-Jennings chart can be overlaid onto a bell-shaped/Gaussian curve to illustrate the overall distribution of quality control values (see Figure 4).

Figure 4: Relative Distribution of QC Values



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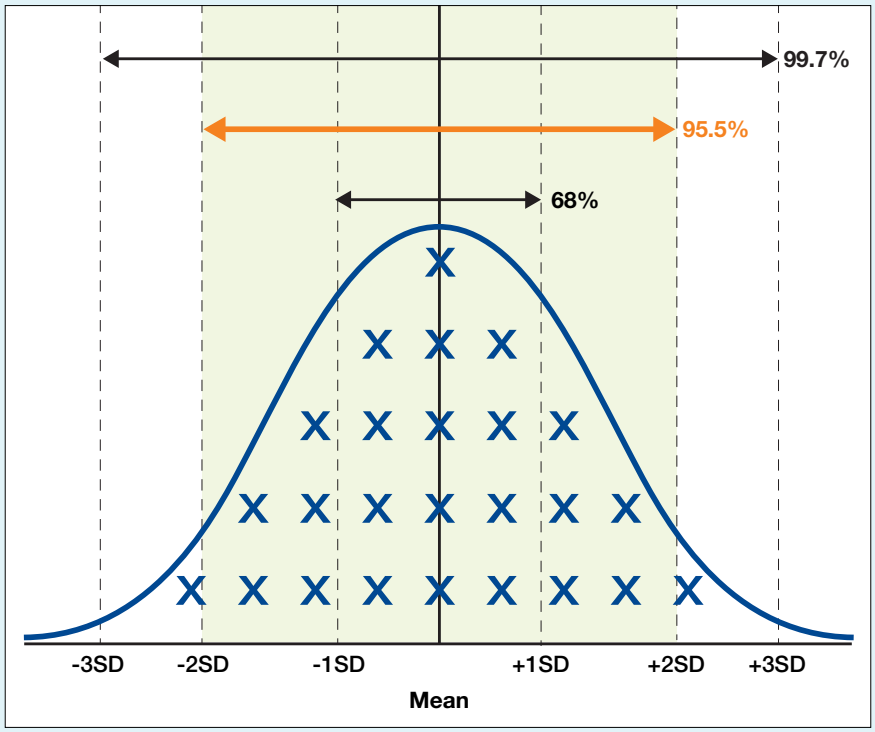
When an analytical process is within control, approximately 68% of all QC values fall within ±1 standard deviation (1s). Likewise 95.5% of all QC values fall within ±2 standard deviations (2s) of the mean. About 4.5% of all data will be outside the ±2s limits when the analytical process is in control. 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 may be associated with a significant error condition.

Caution

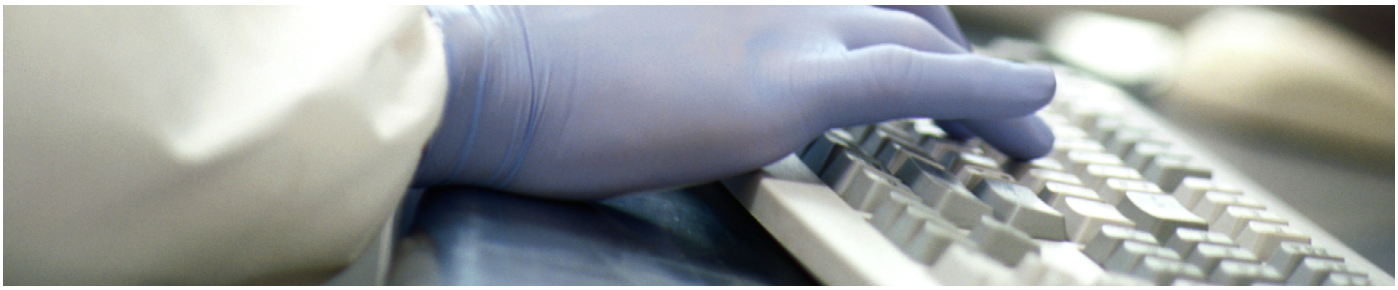
Some laboratories consider any quality control value outside its ±2s limits to be out of control and incorrectly decide that the patient specimens or QC values are invalid. An analytical run may be acceptable if a single quality control value is outside the ±2s QC limits but within the ±3s QC limits.

Approximately 4.5% of all valid QC values will fall outside +/- 2 standard deviation limits (see schematic representation below) Laboratories that use a ±2s limit frequently reject good runs. That means patient samples are repeated unnecessarily, labor and materials are wasted, and patient results are unnecessarily delayed.

The combination of patient and quality control specimens analyzed together is referred to as an "analytical run" or "run" for short.



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Using a Levey-Jennings Chart to Evaluate Run Quality

The laboratory needs to document that quality control materials are tested 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 in QC data management software. 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. Optional items for the log include: method and the assay temperature (usually included for enzymes). There should be room to enter corrective actions taken to resolve any situation which is identified as “out-of-control” or unacceptable and a place for documentation of supervisory review.

Once the QC results are entered into the QC Log, they should be plotted on the Levey-Jennings chart. When the results are plotted, an assessment can be made about the quality of the run. The laboratory professional performing the test should look for systematic error and random error.

Systematic Error

Systematic error is evidenced by a change in the mean of the control values. 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.

Trend

A trend indicates a gradual loss of reliability in the test system. Trends are usually subtle. Causes of trending may include:

- Deterioration of the instrument light source
- Gradual accumulation of debris in sample/reagent tubing
- Gradual accumulation of debris on electrode surfaces
- Aging of reagents
- Gradual deterioration of control materials
- Gradual deterioration of incubation chamber temperature (enzymes only)
- Gradual deterioration of light filter integrity
- Gradual deterioration of calibration

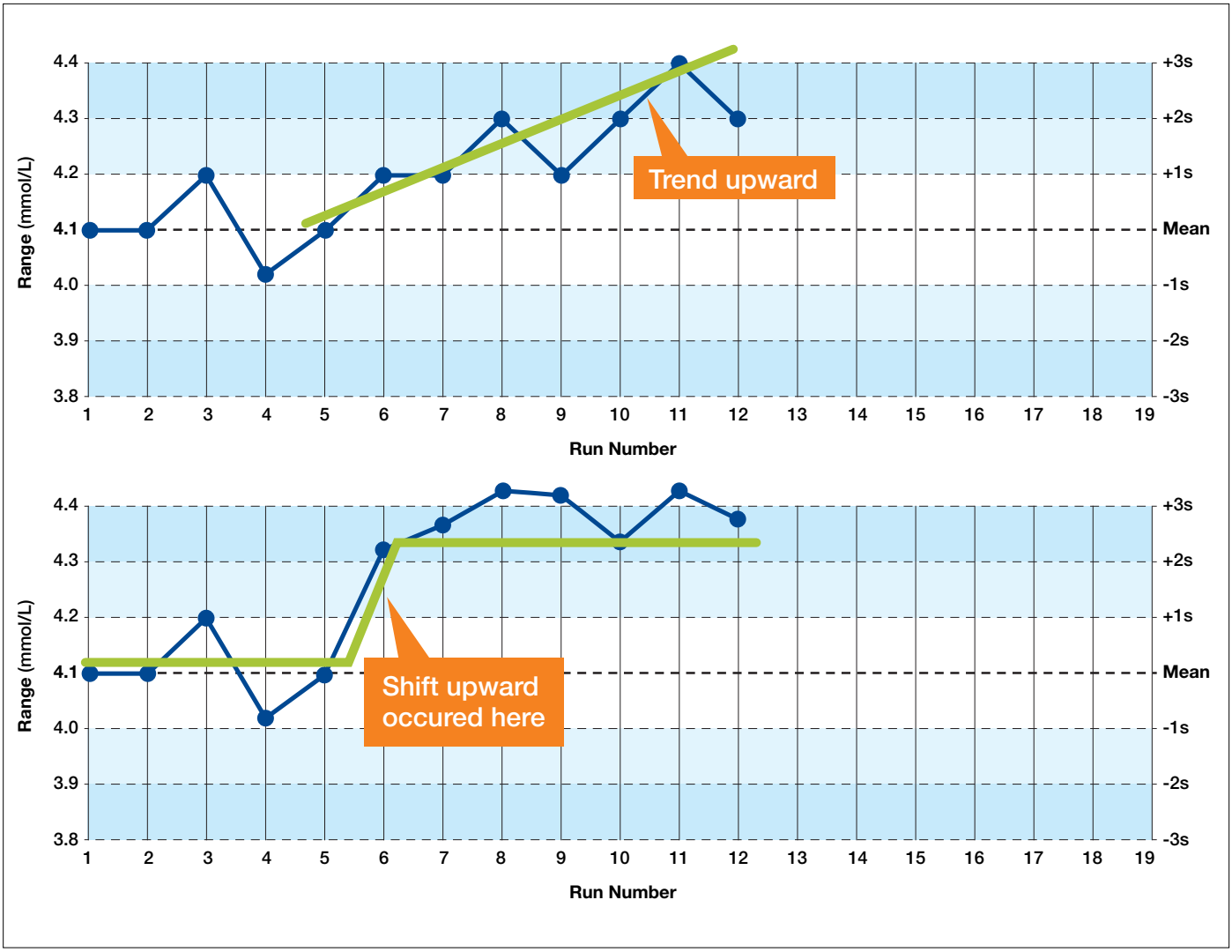
Shift

Abrupt changes in the control mean are defined as shifts. Shifts in QC data represent a sudden and dramatic positive or negative change in test system performance. Shifts may be caused by:

- Sudden failure or change in the light source
- 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 dispensing system
- Inaccurate calibration/recalibration

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Figure 5: Example of Trend and Shift



Random Error

Technically, random error is any deviation away from an expected result. For QC results, any positive or negative deviation away from the calculated mean is defined as random error. There is acceptable (or expected) random error as defined and quantified by standard deviation. There is unacceptable (unexpected) random error that is any data point outside the expected population of data (e.g., a data point outside the ±3s limits).

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Quality Control Statistical Rules

Statistical process controls ('QC rules') are the primary method for assessing if a test method is operating normally ('in control'). The control materials are tested and the results are evaluated with the designated QC rule to determine if the control run is accepted or rejected. There are some basic statistical rules frequently used.[7]

These rules are used individually or in combination to evaluate the quality of analytical runs. Most of the quality control rules can be expressed as N_L 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} represents a control rule that is violated when one control observation exceeds the ±3s control limits.[8] The specific QC rule (or rules) to be used for a test method is selected based on the performance of the test method with respect to its quality specification.

[7]Also referred to as Westgard rules.

[8]The visual representation of these rules can sometimes be slightly different, depending on the software or format used (e.g., 1:3s, 1-3s, 1 3s, 13s).

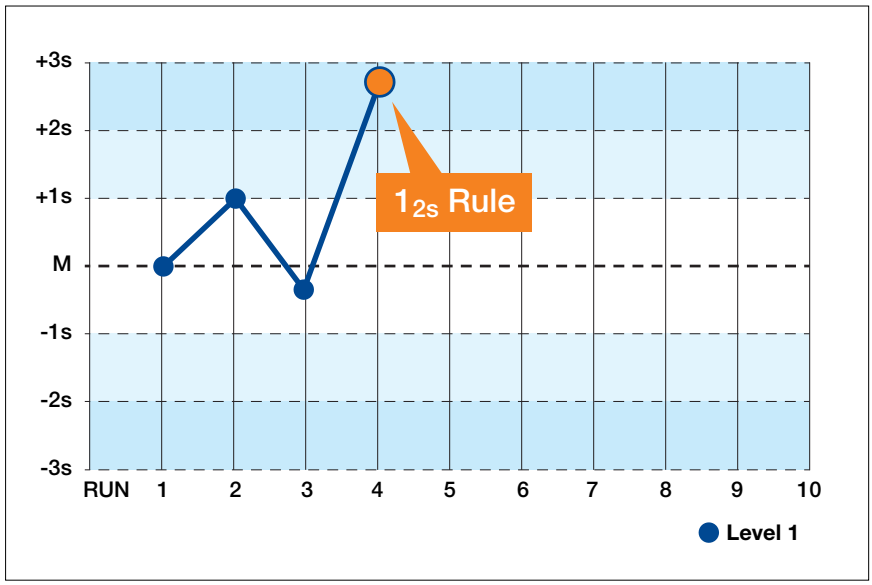
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Rule 1_{2S}

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.

Caution: using the 1_{2S} rule as a rejection rule will generate 4.5% false rejections.

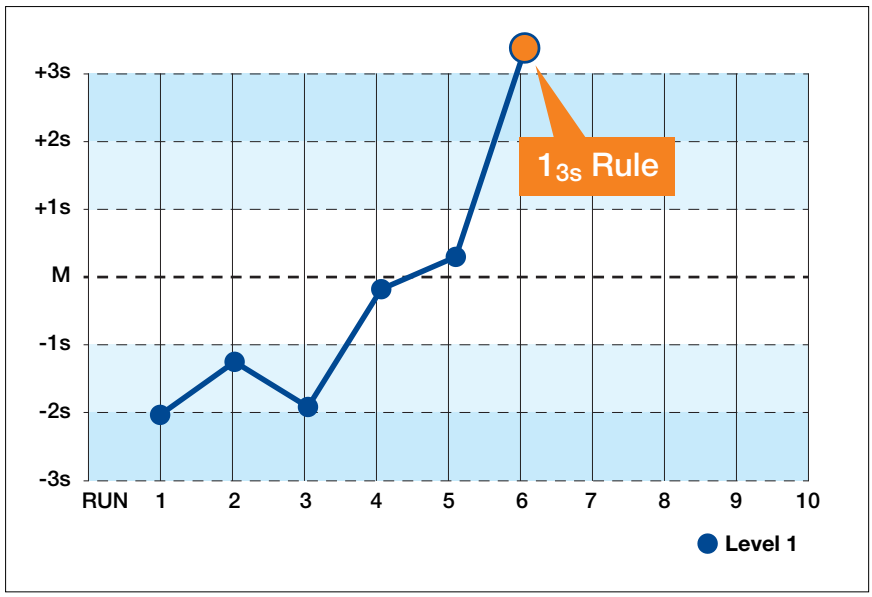
Figure 6: 1_{2S} Rule



Rule 1_{3S}

This rule identifies unacceptable random error or possibly the beginning of a large systematic error. Any QC result outside ±3s violates this rule and should be rejected.

Figure 7: 1_{3S} Rule



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2_{2S} Rule

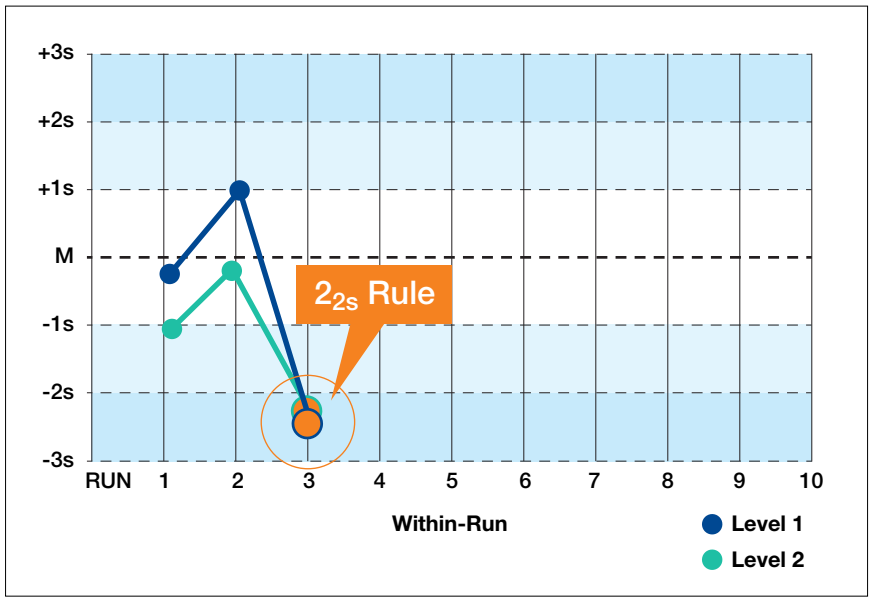
This rule identifies systematic error only. The criteria for violation of this rule are:

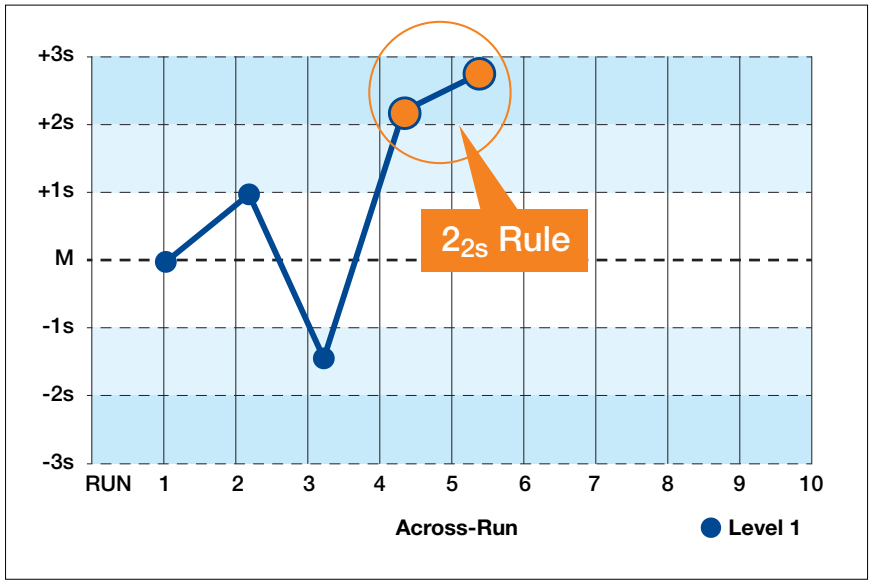
- Two consecutive QC results
- Greater than 2s
- On the same side of the mean

There are two applications to this rule: within-run and across-runs. The within-run application affects all control results obtained for the current analytical run. For example, if a normal (level 1) and abnormal (level 2) 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. The across-run application of this rule affects only 1 control level. If the current and previous run are both greater than 2s the across-run application for systematical error is violated and the results should be rejected.

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 8: 2_{2S} Rule



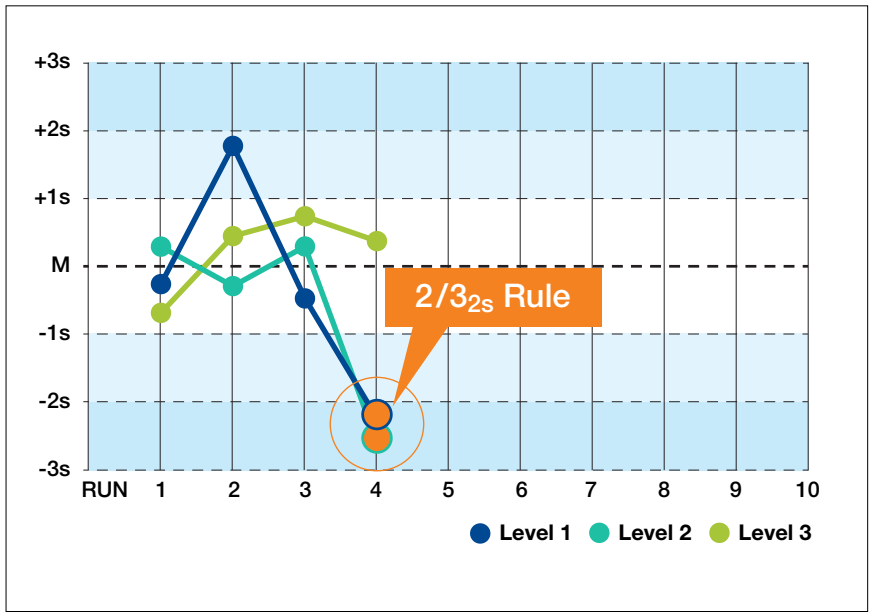


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$2/3_{2S}$ Rule

For three level controls the 2_{2s} rule is also expressed as $2/3_{2s}$. Whenever any two of the three levels violate the criteria for this rule within the run, unacceptable systematic error may be present and must be resolved.

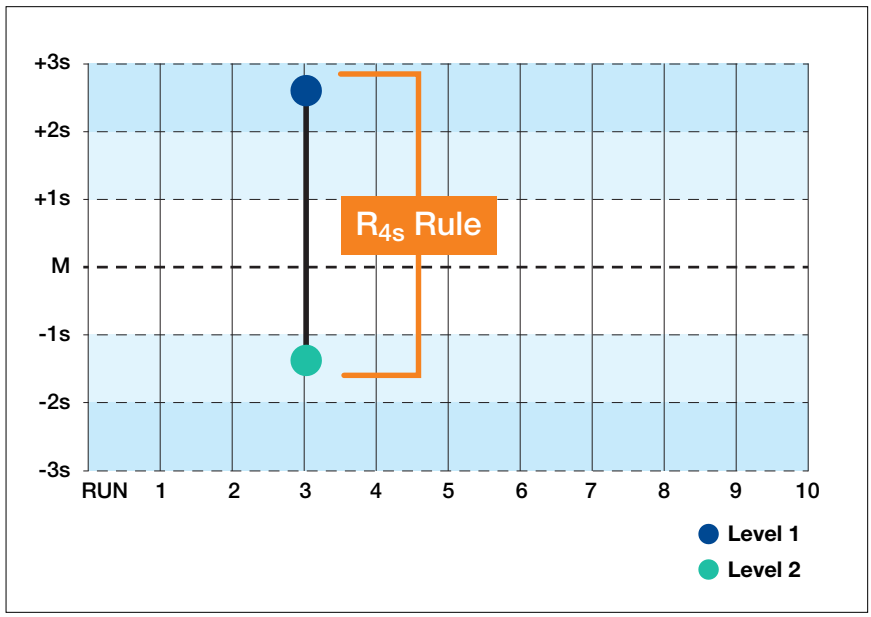
Figure 9: $2/3_{2S}$ Rule



R_{4S} Rule

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 1 and level 2have been assayed within the current run. Level 1 is +2.8s above the mean and level 2 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).

Figure 10: R_{4S} Rule



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Violation of any of the following rules does not necessarily require rejection of the analytical run. These violations typically identify smaller systematic error or analytical bias that is not often clinically significant or relevant. Analytical bias may be eliminated by performing calibration or instrument maintenance.

3_{1S} Rule

The criteria which must be met to violate this rule are:

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

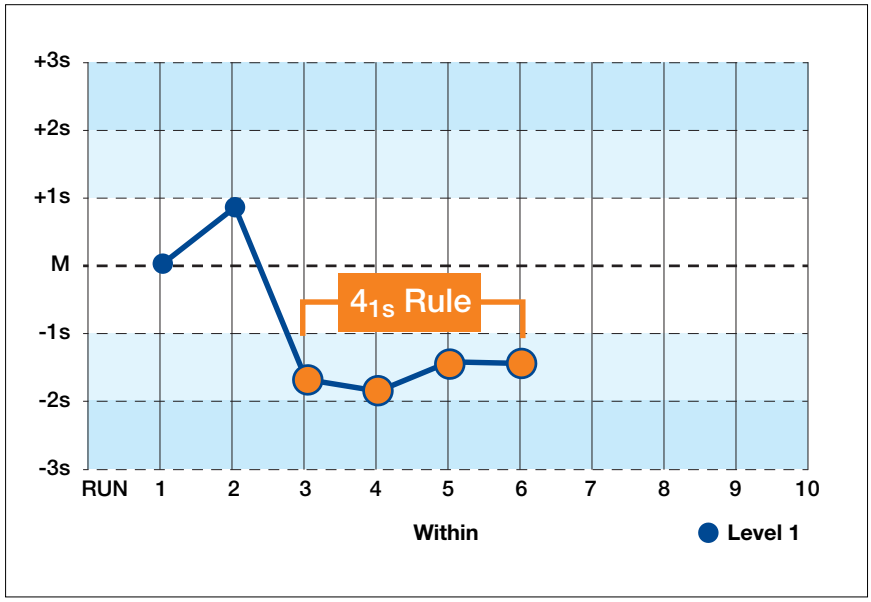
4_{1S} Rule

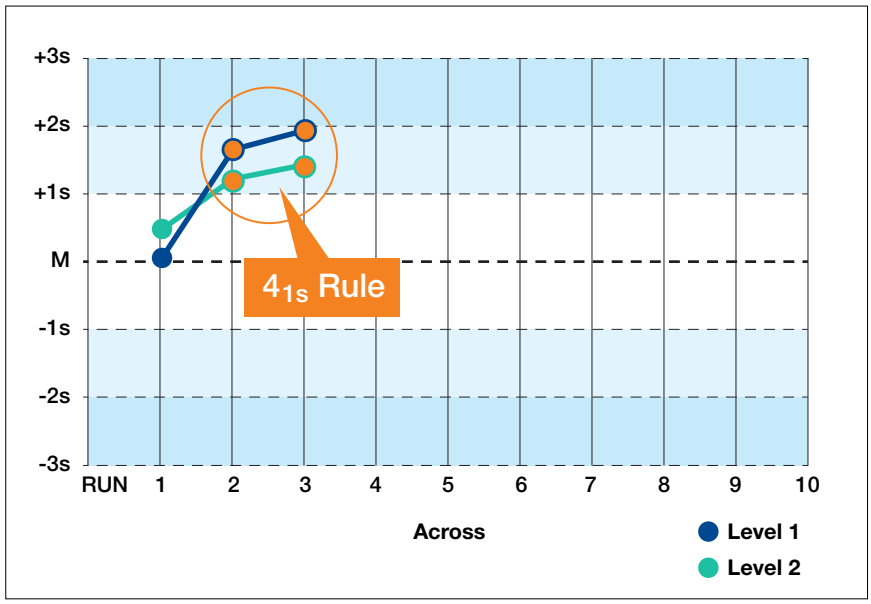
The criteria which must be met to violate this rule are:

- 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 1 control results) or across control materials (e.g., level 1, 2, and 3 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 error over a broader concentration.[9]

Figure 11: 4_{1S} Rule





[9]Use of 3_{1S} detects smaller analytical bias than 41s and is said to be more sensitive to analytical bias.

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Rules 7$\bar{x}$, 8$\bar{x}$, 9$\bar{x}$, 10$\bar{x}$, and 12$\bar{x}$

These rules are violated when there are:

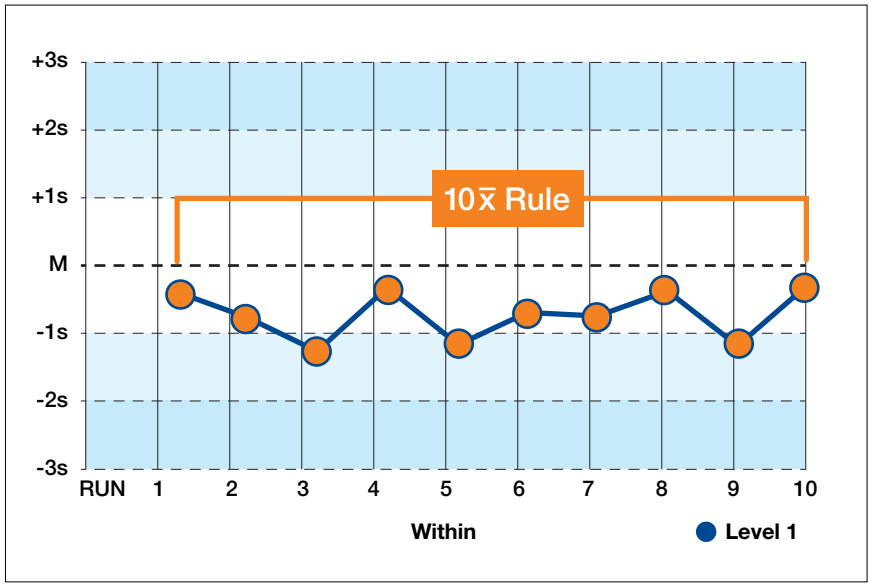
- 7 or 8, or 9, or 10, or 12 control results
- On the same side of the mean regardless of the specific standard deviation in which they are located.

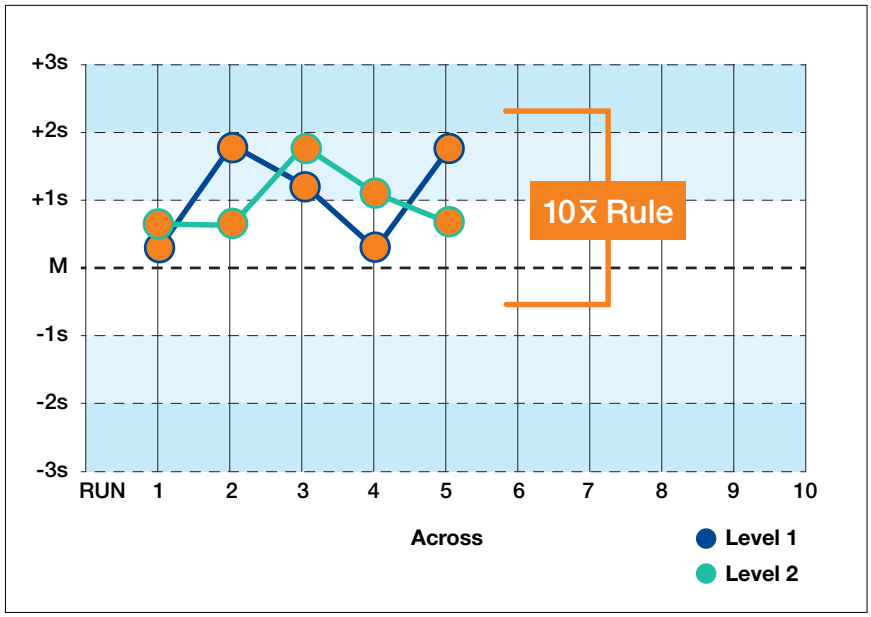
Each of these rules also has two applications:

- Within control material (*e.g.,* all level 1 control results) or across control materials (*e.g.,* Llevel 1, 2, and 3 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 over a broader concentration.

The 7$\bar{x}$ control rule is far more sensitive to analytical bias than the 12$\bar{x}$ and the chances of finding seven consecutive control observations on one side of the mean are much higher than finding twelve. It is extremely important that each individual laboratory be aware of highly sensitive rules like 7$\bar{x}$, 8$\bar{x}$ and 9$\bar{x}$ and apply them sparingly, if at all.

Figure 12: 10$\bar{x}$ Rule



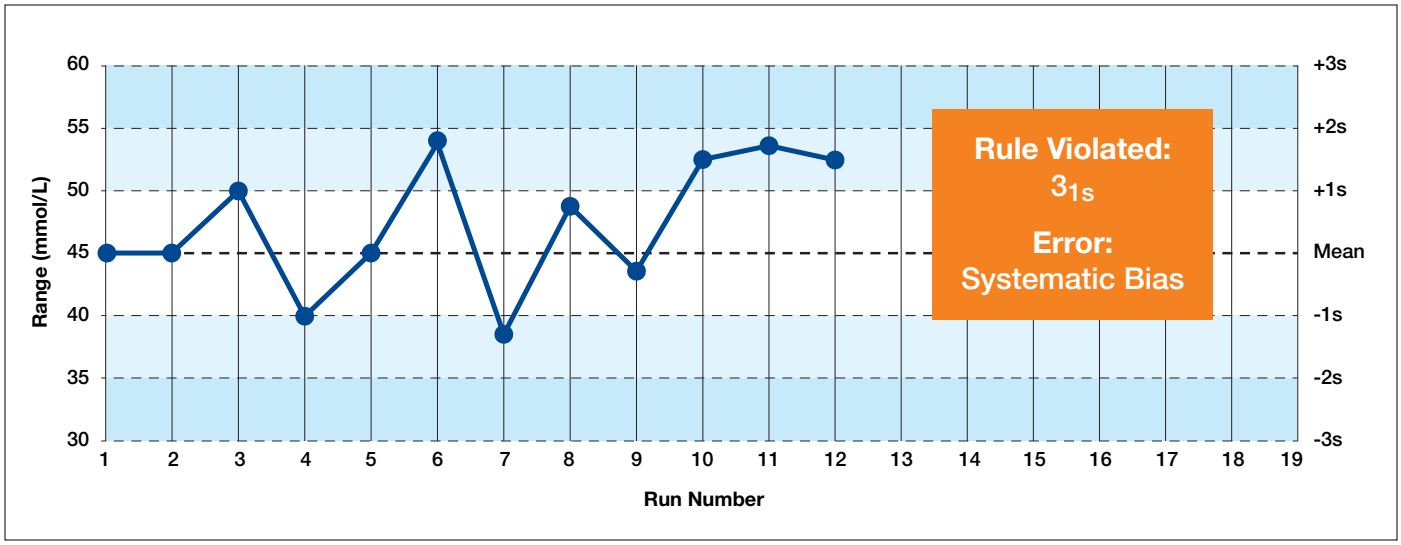


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Practice: Evaluate Levey-Jennings Charts

Study the following Levey-Jennings charts to evaluate the last run (Run Number 12) on each chart. Identify the control rule violated, if any, and the type of error most likely associated with the control rule violations from the multiple choice list (i.e. systematic or random error). Click the button to display the correct answer.

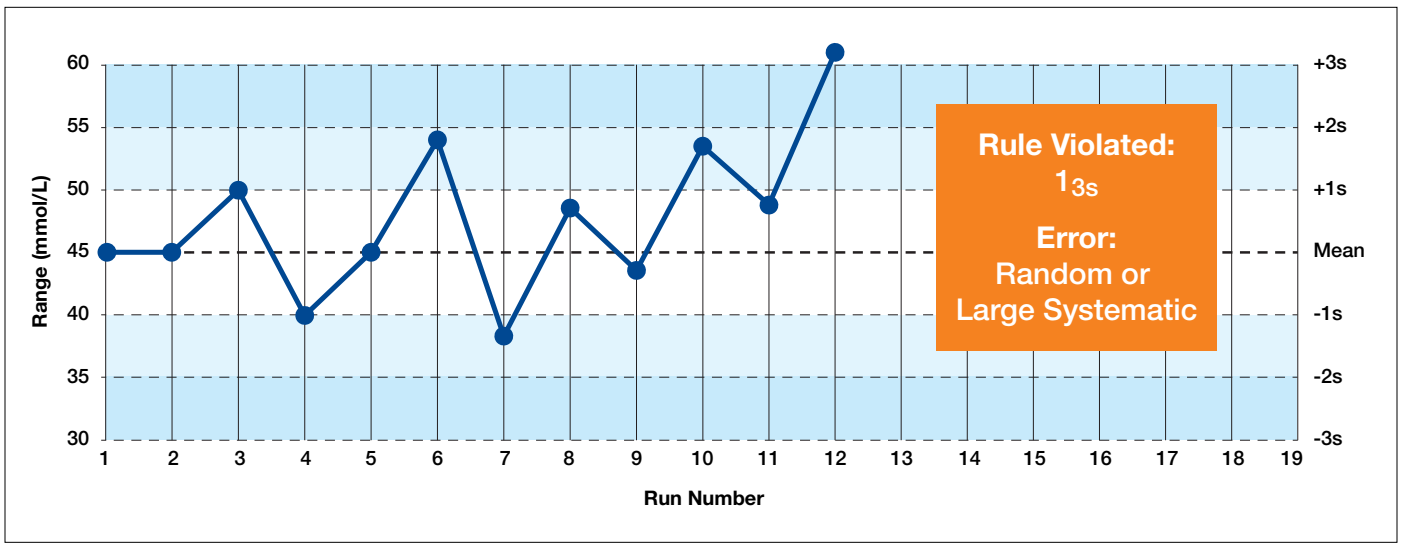
Practice Chart 1: Single Level of Control



Rule Violated:
a) 3_{1s}
b) 1_{3s}
c) 1_{2s}

Error:
a) Warning
b) Systematic Bias
c) Random

Practice Chart 2: Single Level of Control

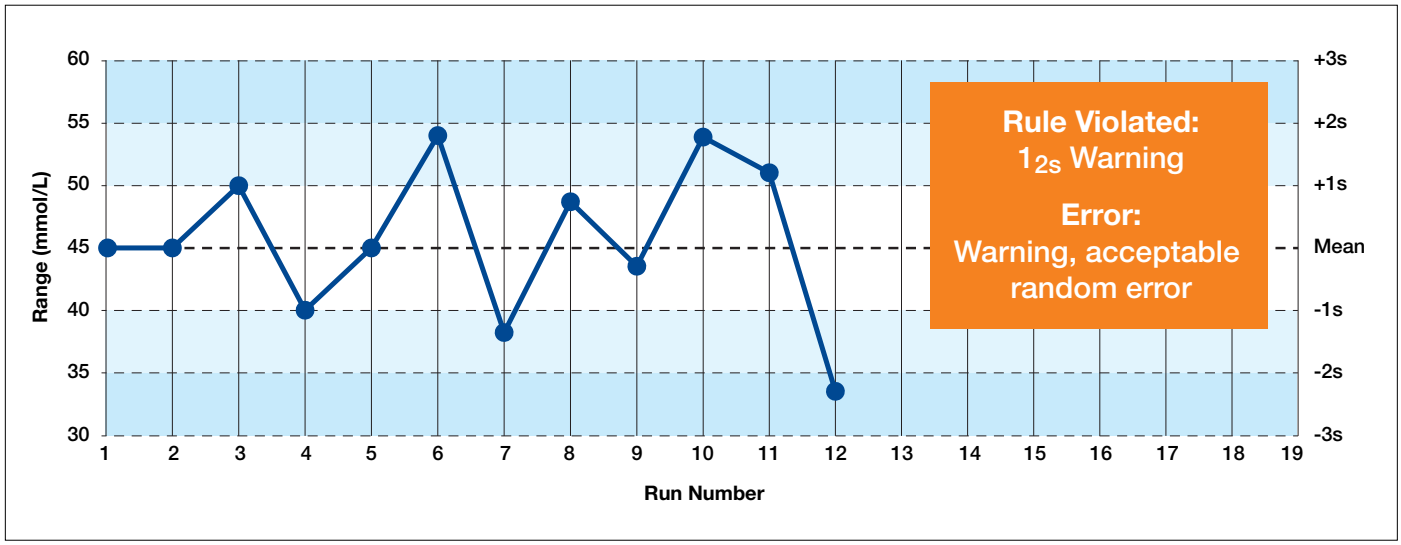


Rule Violated:
a) 3_{1s}
b) 1_{3s}
c) 1_{2s}

Error:
a) Random or Large Systematic
b) Warning
c) Systematic

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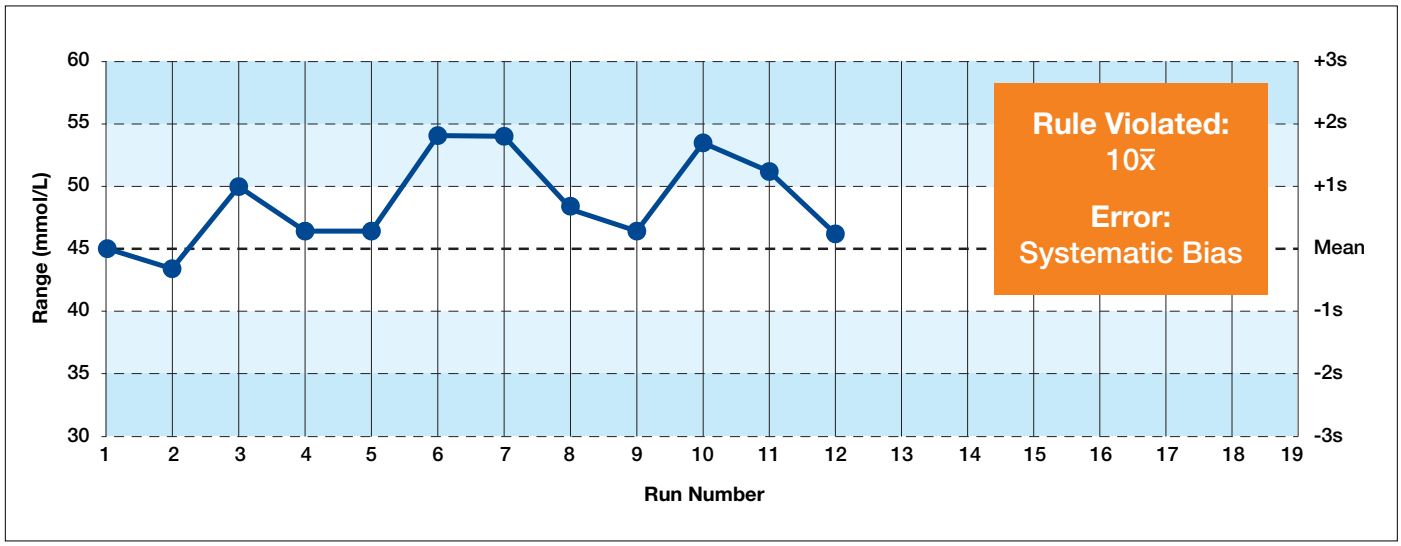
Practice Chart 3: Single Level of Control



Rule Violated:
a) 1_{2s} Warning
b) 1_{3s}
c) R_{4s}

Error:
a) Random
b) Systematic
c) Warning, acceptable random error

Practice Chart 4: Single Level of Control

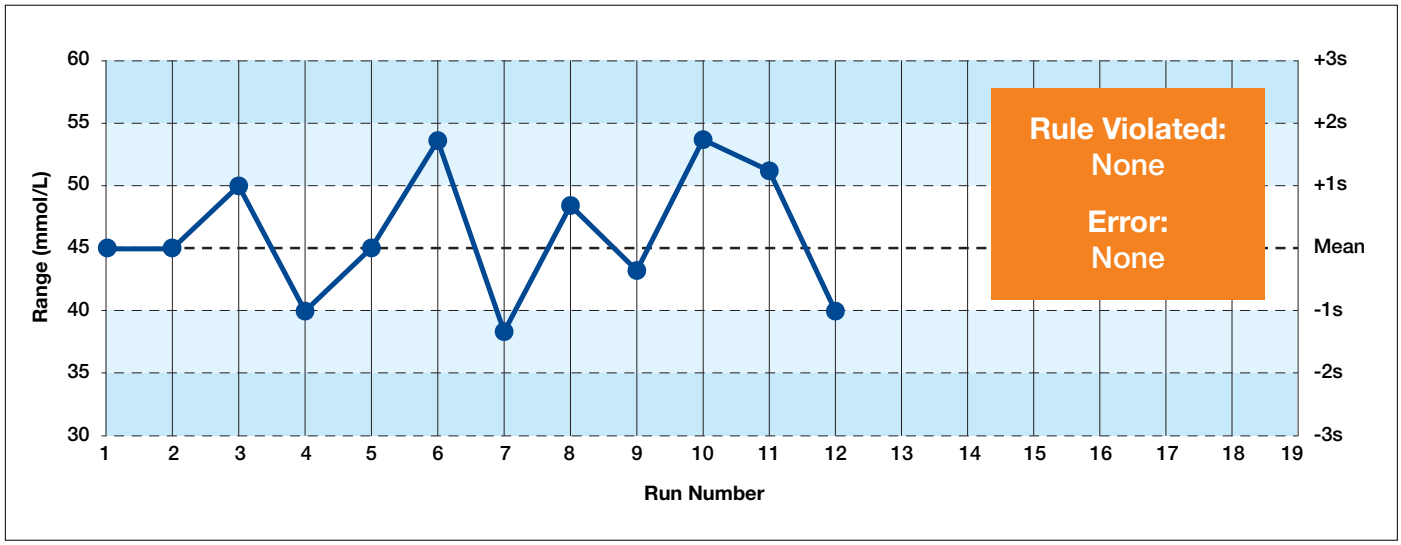


Rule Violated:
a) $10\bar{x}$
b) $7\bar{x}$
c) 1_{3s}

Error:
a) Random
b) Systematic Bias
c) None

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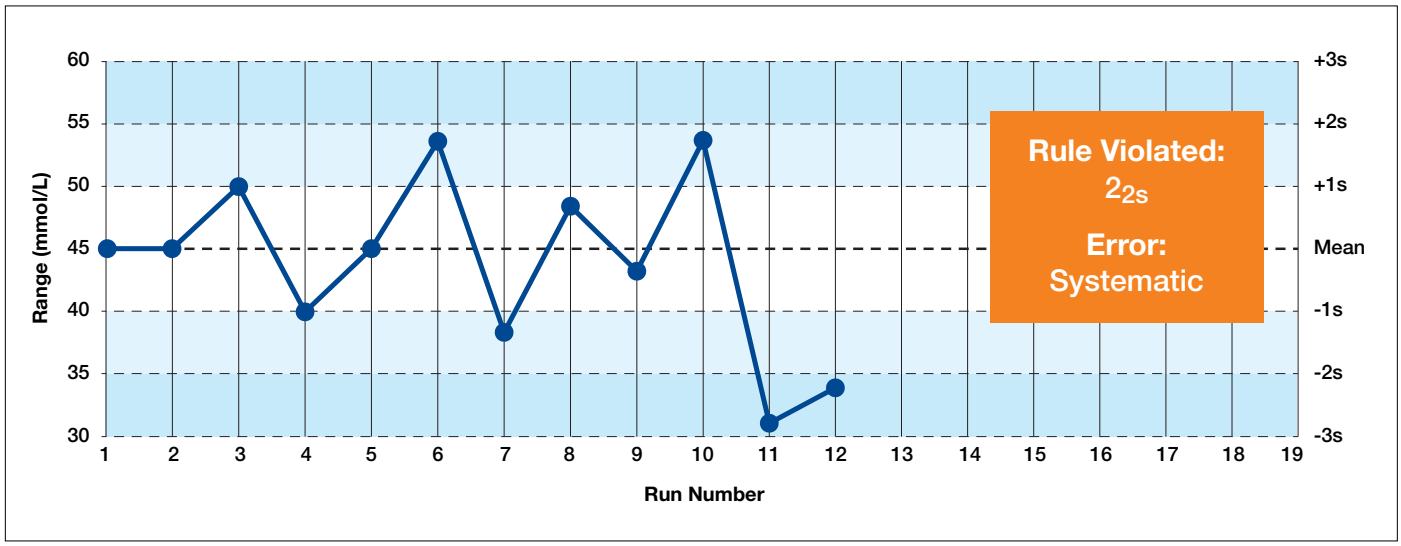
Practice Chart 5: Single Level of Control



Rule Violated:
a) None
b) 1_{3s}
c) 1_{2s}

Error:
a) None
b) Systematic
c) Random

Practice Chart 6: Single Level of Control

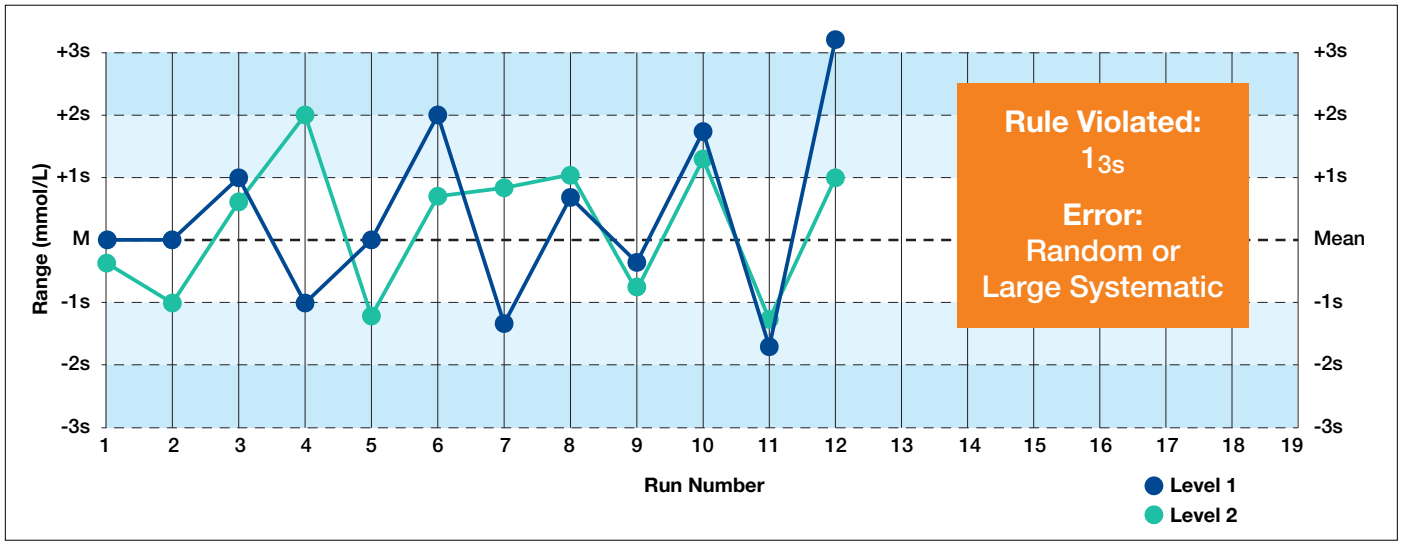


Rule Violated:
a) 1_{2s} Warning
b) 2_{2s}
c) 1_{2s}

Error:
a) Warning, acceptable random error
b) Random
c) Systematic

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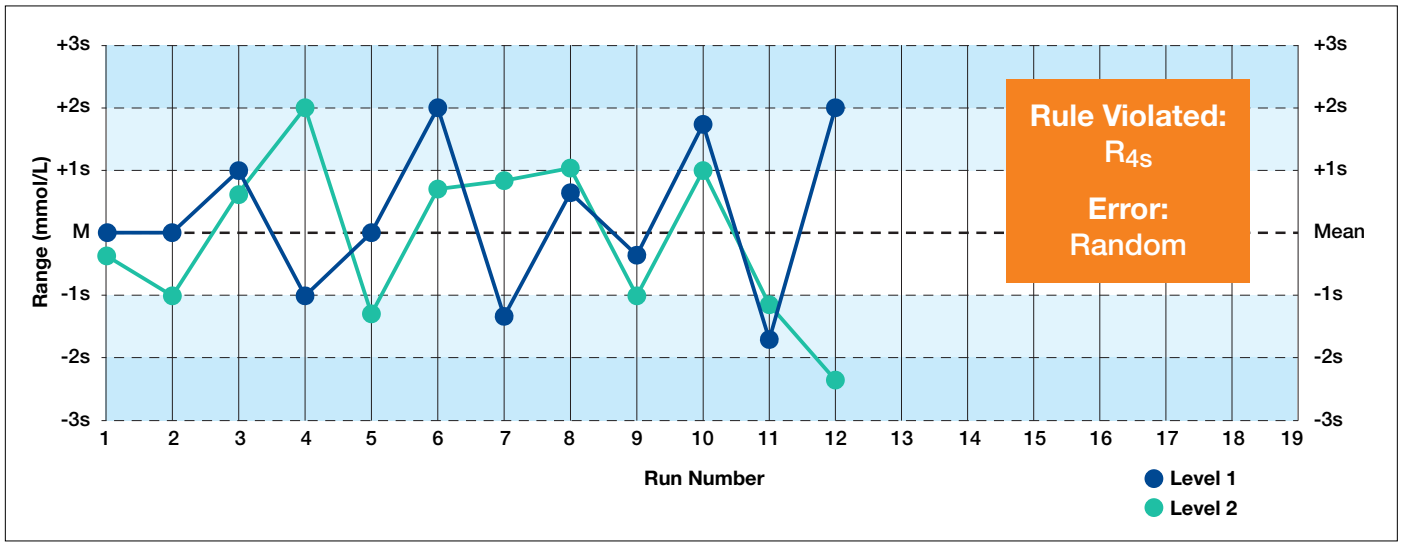
Practice Chart 7: Bilevel Controls (Two Control Levels)



Rule Violated:
a) 1_{3s}
b) 2_{2s}
c) R_{4s}

Error:
a) Random
b) Random or Large Systematic
c) Systematic

Practice Chart 8: Bilevel Controls (Two Control Levels)

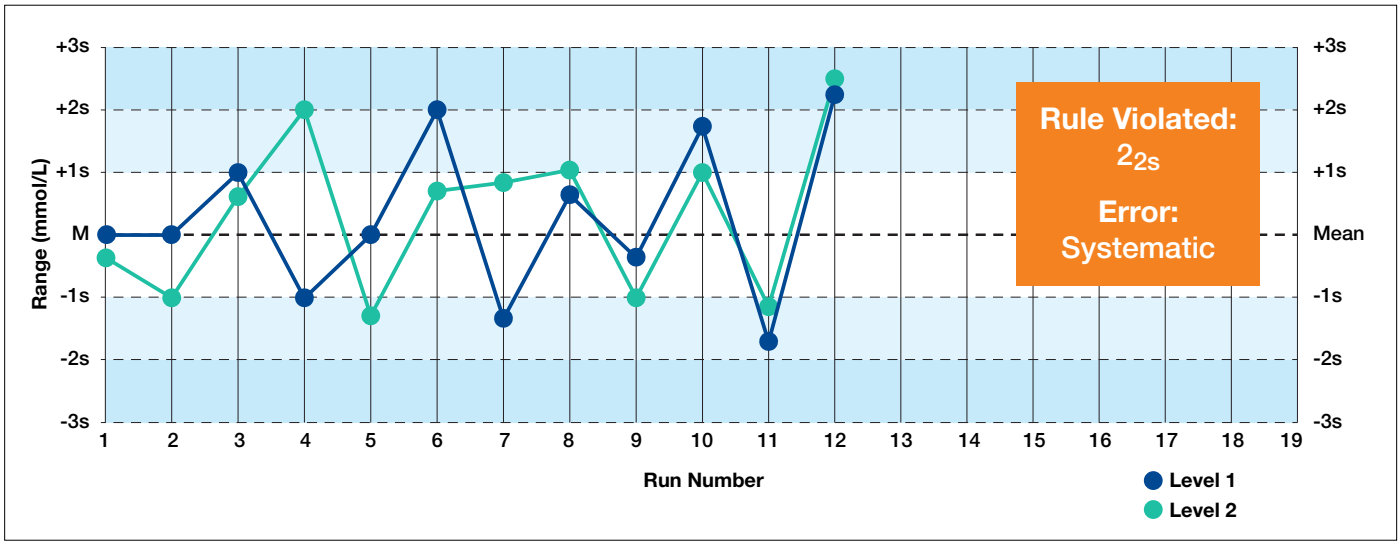


Rule Violated:
a) 1_{3s}
b) 2_{2s}
c) R_{4s}

Error:
a) Random
b) Systematic
c) Random or Large Systematic

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Practice Chart 9: Bilevel Controls (Two Control Levels)



Rule Violated:
a) 1_{3s}
b) 2_{2s}
c) R_{4s}

Error:
a) Random
b) Random or Large Systematic
c) Systematic

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Comparative Evaluations

There are several sources that provide performance expectations to which the laboratory can compare its standard deviation. These include the instrument manual or test method description, proficiency surveys and interlaboratory QC programs.

Instrument Manuals and Test Method Descriptions

Instrument manuals and test method descriptions publish expectations for between-run and within-run precision. These expectations are determined by the manufacturer through repetitive testing and may reflect ideal conditions. If the method description defines a between-run precision of 0.1 mmol/L for potassium, then the laboratory performance from the Table 1 example (0.082; see page 4) meets manufacturer specifications. If however, the between-run specification from the method description is 0.05 mmol/L, then the standard deviation calculated for the example indicates that the laboratory is less precise than the manufacturer's expectation. This may indicate a possible problem exists. However, before any final assessment is made, the laboratory should compare its results to proficiency and/ or interlaboratory QC reports which are more indicative of "real world" experience.

Proficiency Surveys

Laboratories participating in a proficiency testing program[10] receive a set of "unknown" liquid or lyophilized samples. The samples are assayed by the laboratory for each test performed. Results are obtained and reported to the proficiency provider. The provider collects the data and, using various statistical models, determines what the consensus value of the unknown sample should be for each test. Then, the test result reported by each laboratory is compared to this consensus value and the laboratory is graded for accuracy.

The proficiency provider returns a summary report that contains summary data of all the participating laboratories along with a grading report. The summary report identifies, among other statistics, the standard deviation of all values submitted by participating laboratories for each test. This statistic can be used to compare and assess day-to-day laboratory precision. The same type of information can be obtained from interlaboratory comparison reports supplied by most Quality Control manufacturers.

[10]Proficiency regulations vary widely from country to country.

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Interlaboratory QC Programs

In an interlaboratory comparison program, laboratories submit monthly data collected for each quality control product tested. These data are combined with data from other laboratories which use the same instrument.[11] The benefit of an interlaboratory program over a proficiency program is that the interlaboratory program provides statistics collected from repeated daily testing, as opposed to single events that may occur only a few times per year.

The mean of the laboratory is compared with the comparison group (Peers, Method, all labs). The difference between the mean of the laboratory and the comparison group is the bias, which assesses the trueness of the test. The interlaboratory comparison program is fully complementary of any proficiency testing program.

Coefficient of Variation Ratio (CVR)

Although trueness of test results is paramount in the clinical laboratory, precision is just as important. One way a laboratory can determine whether the precision of a specific test is acceptable is to compare its precision to that of another laboratory performing the same test on the same instrument using the same reagents (laboratory peer group).

Formula 5: Calculating the Coefficient of Variation Rate (CVR)

$$CVR = \frac{\text{Within Laboratory CV}}{\text{Peer Group CV}}$$

An easy way to make this comparison is to divide the laboratory CV by the laboratory peer group CV obtained from an interlaboratory comparison report.

For example, if the CV for potassium on a particular instrument is 4% and the potassium for all other laboratories using the same instrument is 4.2%, then the coefficient of variation ratio [CVR] is 4/4.2 or 0.95. Any ratio less than 1.0 indicates that precision is better than the peer group. Any score greater than 1.0 indicates that imprecision is larger. Ratios greater than 1.5 indicate a need to investigate the cause of imprecision and any ratio greater than 2.0 indicates need for troubleshooting and corrective action. Something in the test system is causing the increased imprecision and patient test results may not be entirely reliable. Repeated tests such as glucose for diabetic patients or prothrombin times for patients taking coumadin cannot be considered reliable when the imprecision is high.

[11]A few interlaboratory comparison programs (*e.g.* Bio-Rad's Unity Interlaboratory Program) group data by method.

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Standard Deviation Index (SDI])

The standard deviation index (SDI) is a peer-based estimate of reliability. If the peer group mean is defined as $\bar{x}_{Group}$, the standard deviation of the group is defined as s_{Group} and the laboratory's mean is defined as $\bar{x}_{Lab}$ (see Formula 6).

Formula 6: Calculating the Standard Deviation Index (SDI)

$$SDI = \frac{(\bar{x}_{Lab} - \bar{x}_{Group})}{s_{Group}}$$

The target SDI is 0.0 which indicates a perfect comparison with the peer group. The following guidelines may be used with SDI. A value of:

- 1.49 or less is considered acceptable.
- 1.5 – 1.99 is considered lower performance and investigation of the test system is recommended.
- 2.0 or greater is generally considered to be unacceptable performance and remedial action is usually required.

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Practice: Calculating the Coefficient of Variation Ratio (CVR) and the Standard Deviation Index (SDI)

Study the following sample test result information, then calculate the CVR and SDI based on the data sets provided. Select your answer from the multiple choice list , then click the button to display the correct answer.

Laboratory A

Level 1 (Normal Control)

Chemistry Control, Lot No. 12345
Test: Creatine Kinase
Instrument: ABC
Units: U/L

Control Values

{94, 93, 97, 95, 95, 100, 100, 99, 100, 99}

Level 2 (Abnormal Control)

Chemistry Control, Lot No. 12345
Test: Creatine Kinase
Instrument: ABC
Units: U/L

Control Values

{327, 325, 321, 323, 315, 308, 304,298, 327, 334}

Laboratory B

Level 1 (Normal Control)

Chemistry Control, Lot No. 12345
Test: Creatine Kinase
Instrument: XYZ
Units: U/L

Control Values

{86, 93, 97, 90, 95, 100, 103, 99, 104, 92}

Level 2 (Abnormal Control)

Chemistry Control, Lot No. 12345
Test: Creatine Kinase
Instrument: XYZ
Units: U/L

Control Values

{342, 325, 321, 323, 315, 298, 288, 298, 327, 350}

Calculating the Coefficient of Variation Ratio

Calculate the CVR for Laboratory A and Laboratory B data sets.
Assume that the peer group CV is 2.5% for level 1 and 3.0% for level 2.

Laboratory A

Level 1 CVR a) 0.35
b) 1.13
c) 4.62

Level 2 CVR a) 1.21
b) 1.85
c) 2.76

CVR Laboratory A
Level 1: 1.13
Level 2: 1.21

Laboratory B

Level 1 CVR a) 1.45
b) 2.41
c) 6.42

Level 2 CVR a) 2.55
b) 2.05
c) 1.38

CVR Laboratory B
Level 1: 2.41
Level 2: 2.05

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Calculating the Standard Deviation Index

Calculate the SDI for Laboratory A and Laboratory B data sets. Provide an evaluation of instrument performance. Assume the peer group mean for the level 1 control is 80 U/L and the peer group standard deviation is 13.5 U/L. The peer group mean for the level 2 control is 350 U/L and the peer group standard deviation is 8.0 U/L.

Laboratory A

Level 1 SDI
a) -0.25
b) +1.27
c) +2.66

Evaluation
a) Acceptable
b) Unacceptable performance, remedial action required
c) Lower performance

Level 2 SDI
a) -3.98
b) +4.01
c) -2.15

Evaluation
a) Unacceptable performance, remedial action required.
b) Acceptable
c) Lower performance and investigation of the test is recommended

Laboratory A

Level 1	Level 2
SDI: +1.27	**SDI** -3.98
Evaluation: Acceptable	**Evaluation:** Unacceptable performance, remedial action required

Laboratory B

Level 1 SDI
a) +1.85
b) +1.18
c) -1.18

Evaluation
a) Unacceptable performance, remedial action required
b) Acceptable
c) Lower performance and investigation of the test is recommended

Level 2 SDI
a) -4.95
b) -3.91
c) +4.96

Evaluation
a) Acceptable
b) Unacceptable performance, remedial action required
c) Lower performance

Laboratory B

Level 1	Level 2
SDI: +1.18	**SDI** -3.91
Evaluation: Acceptable	**Evaluation:** Unacceptable performance, remedial action required

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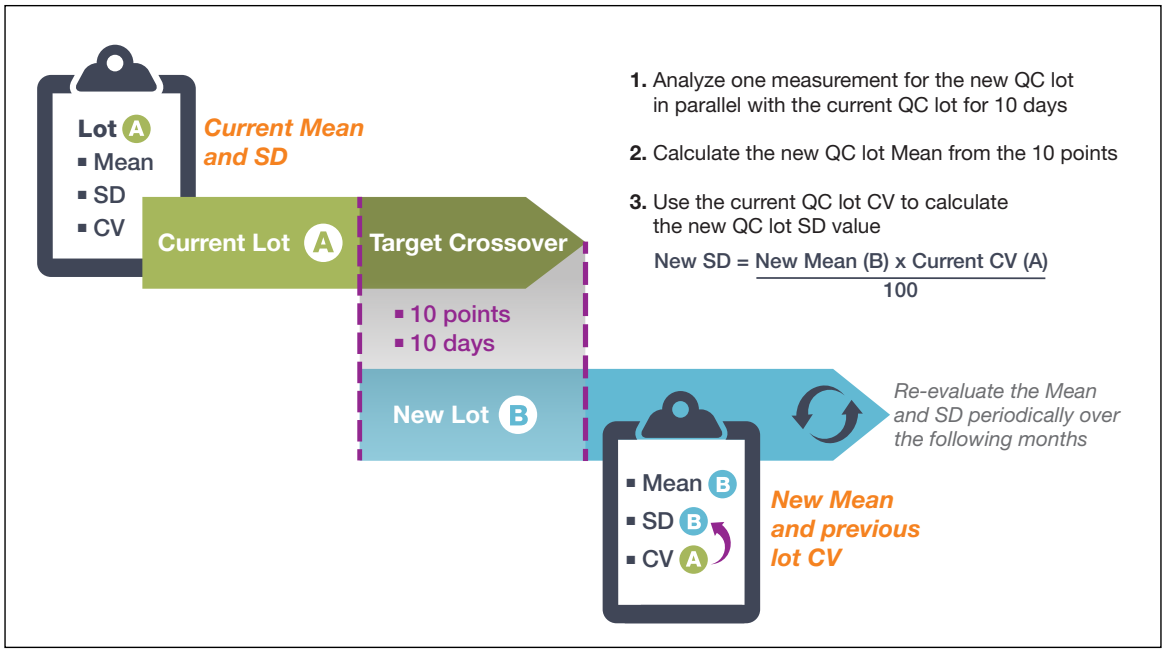
QC Crossover Studies

When a new lot of quality control material is put into use, it should be tested alongside the quality control materials currently in use; *e.g.* the values for the new lot number to be used for calculating the statistics used in statistical process control are verified by the current lot.

In 2016, guidance was updated[12] to allow 10 data points to be used to calculate the mean for a new lot of quality control material. If there is a history of QC data from a period of stable operations of the assay, then the previously established estimate of the SD (or CV) can be use with the new lot of quality control material, provided the new lot is of a similar formulation (manufacturer) and of a similar target concentration. If historic stable estimates are not available, then at least 20 datapoints are required.

In either case, the lab should monitor its QC data over time and update the targets. The additional data provides for a better estimate of SD because of the inclusion of longer-term sources of variability.

Figure 12: Crossover Guidance Chart



Final Examination

When you are ready, click on the link below to take the final test. A certificate of completion will be awarded to anyone who scores 70% or higher. To receive P.A.C.E. credits, please fill out the survey.

Link to Final Examination

Bio-Rad Laboratories is approved as a provider of continuing education in the clinical laboratory sciences by the P.A.C.E. Program through the American Society of Clinical Laboratory Science. This basic to intermediate self instructional course is approved for 2.5 contact hours. This course is also approved for California clinical licensees under the P.A.C.E. California Accrediting Agency License No. 0001.

[12]CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th Edition. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

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Medical Laboratory Vocabulary

The following definitions are offered in layman's terms. Official definitions, often sourced to international standards, can be quite complicated and potentially confusing for an introductory handbook.

A

Accuracy

Closeness of agreement between a measured quantity value and a true quantity value of a measurand (JCGM* 200:2012) An example of an accuracy measurement is a proficiency testing result.

**Joint Commission on Guidance in Metrology*

Across run(s)

A basis for comparing performance between two consecutive runs.

Across control materials

A basis for comparing performance between two different concentrations (Levels) of control material

B

Bias

The difference between an observed value and a known value. For purposes of analytical quality, it is the difference between the average (mean) of a group of values and a known or certified value.

C

Coefficient of Variation (CV)

A statistic that normalizes standard deviation for comparative purposes. It is expressed as a percent. The formula and explanation of coefficient of variation is on page 30.

Coefficient of Variation Ratio (CVR)

A ratio that helps the laboratory to determine whether the precision of a specific test is acceptable. By comparing the laboratory precision to that of another laboratory performing the same test on the same instrument using the same reagents. The formula and explanation of coefficient of variation ratio is on page 29 of the workbook.

Concentration

The amount or quantity of a substance contained within a matrix.

Control materials

See: Quality control materials.

D

Data set

A collection of data within a defined group of data.

E

Error

A mistake or the result of a mistake. Also, the measurable difference between what is expected and what actually occurs or is observed. In a medical laboratory, an error can be inherent and expected as with imprecision (standard deviation) or unexpected as with trends and shifts in performance.

EQA (External Quality Assessment)

The term used to describe a method that allows for comparison of a laboratory's testing to a source outside the laboratory.

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G

Gaussian

Having the shape of a normal curve or a normal distribution.

I

Imprecision

A term that characterizes the lack of agreement among data within a data set. The opposite of precision.

Interlaboratory

Between laboratories.

ISO 15189

International standard of practice for medical laboratories; ISO 15189 Medical laboratories – Requirements for quality and competence.

Intralaboratory

Within an individual laboratory.

L

Levels of control

Control materials of different concentrations.

Levey-Jennings Chart

A graph used to plot quality control data and monitor performance.

Lyophilize(d)

A process used to freeze-dry quality control materials. Lyophilized materials require reconstitution with water before they can be used.

M

Matrix

A substance that contains other constituents. For example, a human matrix such as serum or plasma that contains elements like calcium and potassium and proteins like thyroglobulin.

Mean ($\bar{x}$)

The central value of a data set. That point where half of the values are above and half are below. The best estimate of the true value. The notation for the mean is ($\bar{x}$). The formula and explanation for the mean of a data set is on page 5.

P

Peer Group

A group that shares common characteristics. For statistical process control a peer group consists of laboratories that use the same instrument, reagent, method, and units of measure for a particular test.

Precision

A term that characterizes the closeness of agreement of data within a data set.

Proficiency testing (PT)

A program where an independent, unbiased third party organization sends samples with “unknown” concentrations of measurands for analysis by participating laboratories. Data submitted by participating laboratories are compiled and comparisons are made and reported back to each laboratory. Also known as EQA (External Quality Assessment).

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Q

Quality Control (QC)

A process that uses materials of known value, reaction or performance along with preset parameters, often statistical in nature, to monitor the reliability of medical laboratory testing.

Quality Control Materials

Liquid or freeze-dried (lyophilized) materials that are tested alongside patient samples, once thawed or reconstituted, to verify that analytical performance meets expectations and that results for patient samples are suitable for their intended use. Quality control materials may be made from human materials such as serum, plasma, urine, spinal fluid, manufactured materials made to resemble human fluids or they may be aqueous materials.

R

Run(s)

Discrete cycles of testing patient samples and controls.

Root Cause

An objective process used to identify the "real" or "true" cause of a problem, failure or mistake. More than troubleshooting.

S

Shift

A sudden and unexpected positive or negative change in performance.

Sigma (Σ)

Mathematical notation designating to "take the sum".

Standard deviation (s)

A statistic that characterizes the measure of variability within a data set. Precision. A statistic that quantifies the amount of imprecision present. The closeness of agreement between data points in a data set. Standard deviation is mathematically represented as "s" but the abbreviation "SD" is often used informally in various texts and articles. Both "s" and "SD" are used in this handbook. The formula and explanation for standard deviation is on page 7.

Standard deviation index (SDI)

A statistical measure of bias in a result (or for a group of results) based on standard deviation. The SDI may be positive or negative. The formula and explanation of standard deviation index is on page 30.

Statistical Process Control (SPC)

A process that uses basic statistics such as mean and standard deviation, among others, to construct a framework to monitor the quality of laboratory testing.

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T

Test system

Includes the instrument, reagents, calibrators, sampling mechanism, measurement modules etc.

Trend

A gradual positive or negative change in performance.

Troubleshooting

A series of activities to find the cause of a problem, failure or mistake that typically results in treatment of the symptom but not leading to root cause.

Trueness

The closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value (JCGM 200:2012). See page 29 for an example of a trueness measurement in an interlaboratory QC program.

W

Within-run

A period of time or interval of patient samples between QC events.

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Website *www.bio-rad.com/diagnostics* ***U.S.*** *1 800 224 6723* ***Australia*** *+61 (2) 9914 2800* ***Austria*** *+43 (0) 1 877 89 01 9* ***Belgium*** *+32 (0) 3 710 53 00*
Brazil *+55 11 3065 7550* ***Canada*** *+1 514 334 4372* ***China*** *+86 21 6169 8500* ***Czech Republic*** *+420 241 431 660* ***Denmark*** *+45 44 52 10 00*
Finland *+358 9 804 22 00* ***France*** *+33 (0)1 47 95 60 00* ***Germany*** *+49 (0) 89 31884 393* ***Greece*** *+30 210 7774396* ***Hong Kong*** *+85 2 2789 3300*
Hungary *+36 1 459 6190* ***India*** *+91 124 4029300* ***Israel*** *+972 03 963 6025* ***Italy*** *+39 024 94 86 600* ***Japan*** *+81 3 6361 7070* ***Korea*** *+82 080 007 7373*
Mexico *+52 (55) 5488 7670* ***The Netherlands*** *+31 (0)318 540 666* ***New Zealand*** *+64 (9)415 2280* ***Norway*** *+47 23 38 41 30* ***Poland*** *+48 22 331 99 99*
Portugal *+351 21 47 27 700* ***Russia*** *+7 495 721 1404* ***Singapore*** *+65 6415 3170* ***South Africa*** *+27 11 442 8508* ***Spain*** *+34 91 490 6580*
Sweden *+46 844 98053* ***Switzerland*** *+41 (0) 61 717 9555* ***Taiwan*** *+886 (2) 2578-7189* ***Thailand*** *(662) 651 8311* ***United Kingdom*** *+44(0)1923 471301*

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