

Variation in 1(3S) and 2(2S) rejections incidence in internal quality control program on using 20 reading mean and SD of different periods

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Abstract

Objectives: Objective of the study is to measure mean and standard deviation of 1(3S) and 2(2S) rejections in six month period using consecutive 20 readings of different periods of internal quality control program.

Material & Method: Measurement of Glucose, Bilirubin, ALT, Creatinine, Na⁺, K⁺, Urea, Total protein, Albumin in two level quality control sera for two hundred days twice daily was conducted.

Result & Discussion: Of 10 parameters' Quality control results analyzed, it is observed that there is wide variation in number of time 2(2S) and 1(3S) rules broken, depending on which slot of 20 values are used for calculation of mean and SD. While some analyte like direct bilirubin shows variation in rejection rate with 2(2S) QC rules from 0-1.9% of observation at 95% confidence, other analyte like K⁺ shows variation in rejection rate with 2(2S) QC rules from 0-24.3% of observation at 95% confidence. Higher variation in rejection rate for Na⁺, K⁺, ALT and total protein signify that, use of 20 reading SD and mean can result in extreme of false sense of high quality at times and high rate of false rejection at other times.

Conclusion: The high variation in observed QC rejections indicate that, 20 sample size, advised by CLSI C24-A2 is too small to be useful, as it may result in over-rejection and under-rejection resulting in either too much of failed root-cause analysis with no end result or too much of sense of quality. The major causes for such variations are likely to be Statistical variation in sample mean and SD from population mean and SD, day to day variation in equipment performance, day to day variation in reagent quality and day to day variation in reconstitution of QC materials.

Keywords: Quality Control rules, internal quality control

1. Introduction

Internal Quality Control programme and External Quality Assurance Programme are backbone of quality system of any modern day clinical laboratory. A laboratory, implementing Internal Quality Control program frequency faces situation where there is change in Quality control sera lot, change in method and change in equipment. In such situation laboratory needs to derive newer means and standard deviations for each examination.

Section 8.6.3 of CLSI guideline C24- A2, states that "Establishing the Value of the Mean on a New Lot New lots of control material should be analyzed for each analyte in parallel with the control material in current use. Ideally, a minimum of at least 20 bottles should be assayed on separate days. If the desired 20 data points from 20 days are not available, provisional values may have to be set from fewer than 20 days. Possible approaches include making no more than four control measurements per day for at least five different days." [1]

This study aims to measure variation in rejection of run based on different 20 data points for 2(2S) and 1(3S) rules.

1(3s) rule symbolizes the control rule where a run is rejected when one control observation exceeds control limits set as mean \pm 3s. These are the usual "action" or rejection limits on a Shewhart control chart.

2(2s) rule is the control rule where the run is rejected when two consecutive control observations exceed the same limit, which is either $x + 2s$ or $x - 2s$. The rule is initially applied to the two observations within a run, one on each of two different control materials. The run is rejected when the control observations on both materials exceed their respective $+2s$ control limits or their respective $-2s$ control limits. The rule can also be applied to two consecutive observations on the same control material, one from each of two consecutive runs. When applied to consecutive observations on different materials, this will be referred to as "across" materials, to differentiate this from consecutive observations on the same material, or "within" materials.

2. Materials and methods

2.1 Settings

The new Civil Hospital Surat is a 1150 bed tertiary care academic medical center. The clinical laboratories include Clinical Biochemistry, Clinical pathology, and Histopathology and Microbiology laboratory. In Clinical Biochemistry, the study was performed for following parameters: glucose [GLC], total bilirubin [TBIL], direct bilirubin [DBIL], alanine transaminase [ALT], creatinine [CR], total protein [TP], albumin [ALB], urea [URE], sodium [Na+], potassium [K+]. One high level and one normal level QC sera from Randox, called Human Assayed Control 3 and Human Assayed Control 2 respectively were analyzed three time a day from 16-June-2014 to 28-Feb-2015 for each analyte mentioned above at about 9 am, 3 pm and 10 pm. Total number of QC data available were from 900 to 1200 for various analyst. The results obtained were exported to computer spreadsheet programme. For each consecutive 20 readings of each analyte, mean and SD were calculated. Each set of mean and SD were used to find 2(2S) and 1(3S) rule break incidences. Variations

in rule break incidences were measured by calculating their SD. For a given analyte QC rules were interpreted as follows.

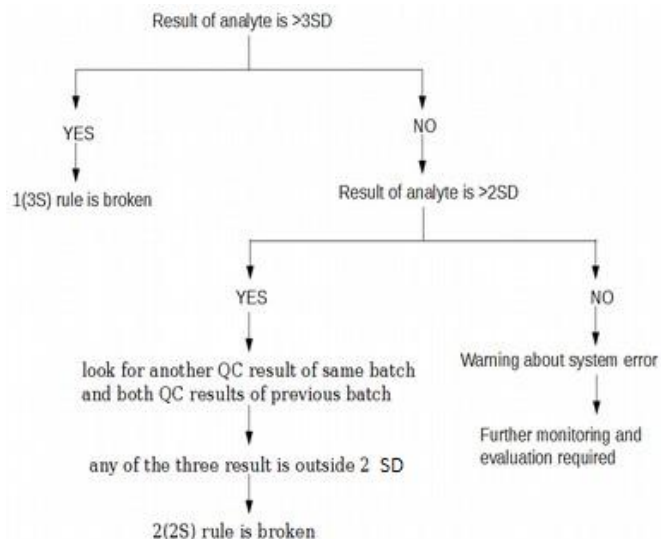


Figure 1: Analyte QC rules interpretation

3. Results

For each analyte spreadsheet was created to find variance in 2(2S) and 1(3S) rule using mean and SD of different slots. Results for various analytes are shown below.

Examination	Total Qc Results for each of normal and Abnormal Qc	Average QC rule break		SD of QC rule break		Average QC rule break in %		SD of QC rule break in %		Limits of variation in % of observation Breaking 1(3S) rules at 95% confidence		Limits of variation in % of observation Breaking 2(2S) rules at 95% confidence	
		1(3s)	2(2s)	1(3s)	2(2s)	1(3s)	2(2s)	1(3s)	2(2s)	Lower limit	Higher limit	Lower limit	Higher limit
ALT	927	49	53	56	48	5.3	5.7	6.0	5.2	0.0	17.4	0.0	16.1
ALB	1001	12	27	13	31	1.2	2.7	1.2	3.1	0.0	3.7	0.0	8.8
GLC	1107	140	84	143	62	12.6	7.6	12.9	5.6	0.0	38.5	0.0	18.7
TBIL	911	12	4	14	8	1.3	0.5	1.5	0.9	0.0	4.3	0.0	2.3
DBIL	934	8	6	5	6	0.8	0.6	0.5	0.6	0.0	1.9	0.0	1.9
CR	1025	26	21	40	22	2.5	2.0	3.9	2.2	0.0	10.3	0.0	6.4
Na	1246	171	94	165	57	13.7	7.5	13.2	4.6	0.0	40.2	0.0	16.7
K	1252	148	83	258	112	19.8	6.6	20.6	8.9	0.0	61.0	0.0	24.5
URE	1052	82	45	69	34	7.8	4.3	6.5	3.2	0.0	16.4	0.0	10.8
TP	923	51	48	50	46	5.5	5.2	5.4	4.9	0.0	15.9	0.0	15.1

The tables above shows wide variation in number of time 2(2S) and 1(3S) rules are broken, depending on which slot of 20 values are used for calculation of mean and SD. While some analyte like direct bilirubin shows variation in rejection rate with 2(2S) QC rules from 0-1.9% of observation at 95% confidence, other analyte like K+ shows variation in rejection rate from with 2(2S) QC rules 0-24.3% of observation at 95% confidence.

In order to find reasons for variation, charts were drawn for number of times QC rules are broken (Y-axis) at each mean and SD observed (X-axis). The results are shown in charts below.

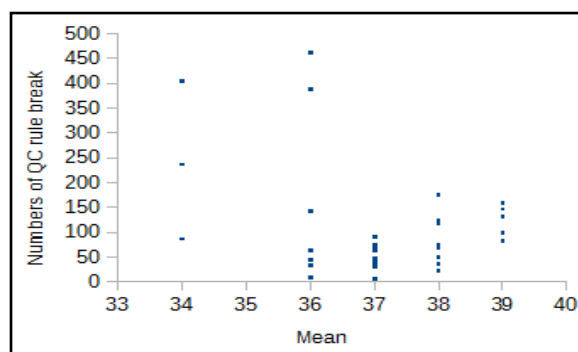


Figure 2: ALT-Total QC Rule break for various mean observed

In above graph for ALT, use of 36 IU/L as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

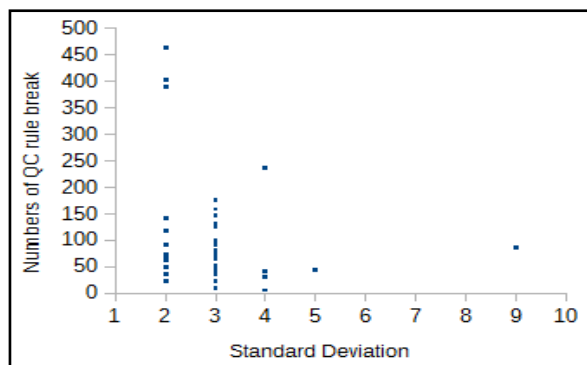


Figure 3: ALT-Total QC Rule break for various SD observed

In above graph for ALT, use of increasing unit as SD, results in decrease in number of QC rule breaks.

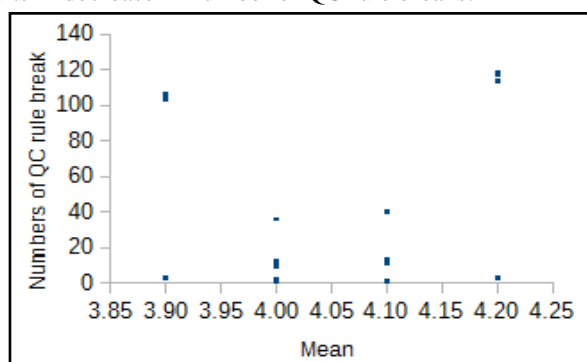


Figure 4: Albumin-Total QC Rule break for various mean observed

In above graph for Albumin, use of 4.0 gm/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks

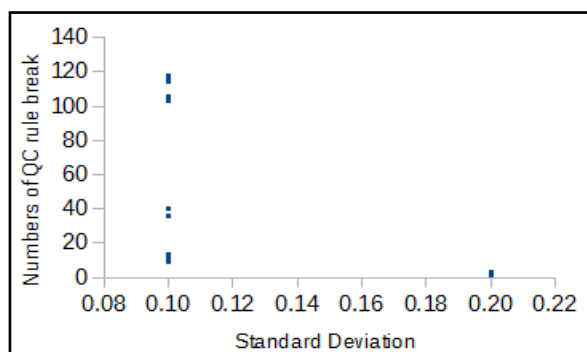


Figure 5: Albumin-Total QC Rule break for various SD observed

In above graph for Albumin, use of 0.2 gm/dl as SD, shows less rule break than 0.1 gm/dl as SD.

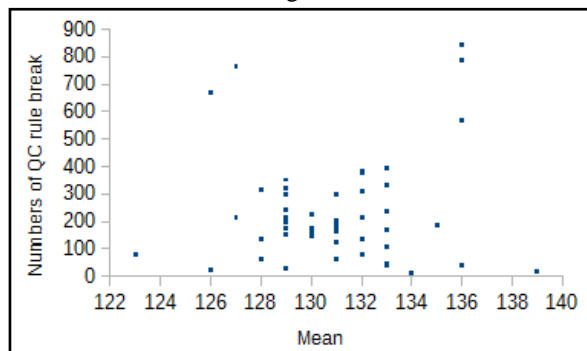


Figure 6: Glucose-Total QC Rule break for various mean observed

In above graph for Glucose, use of 130 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks

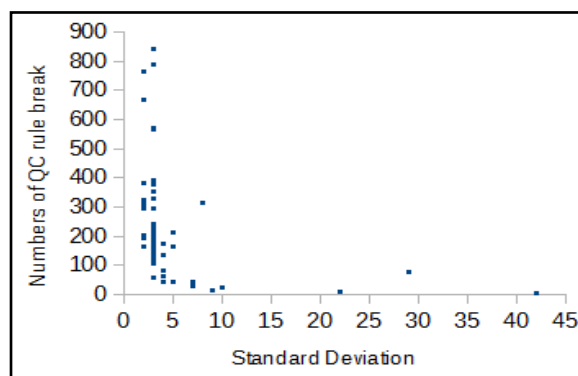


Figure 7: Glucose-Total QC Rule break for various SD observed

In above graph for Glucose, use of increasing unit as SD, results in decrease in number of QC rule breaks.

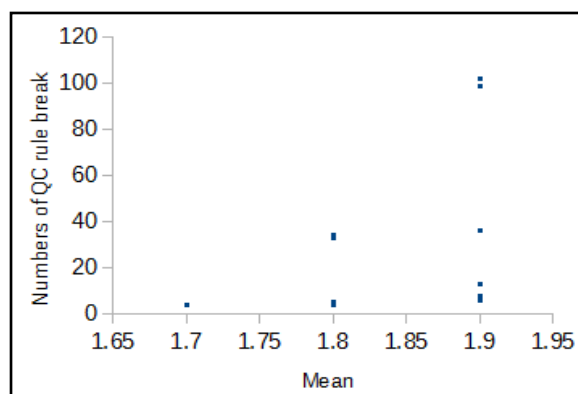


Figure 8: Total Billirubin-Total QC Rule break for various mean observed

In above graph for TBIL, use of 1.7 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks

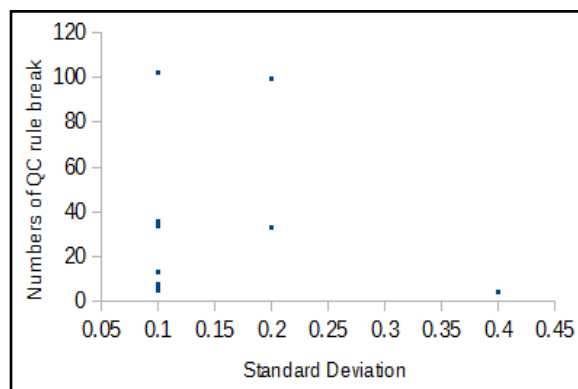


Figure 9: Total Billirubin-Total QC Rule break for various SD observed

In above graph for TBIL, use of 0.2 mg/dl, 0.3 mg/dl and 0.4 mg/dl as SD, results in decrease in number of QC rule breaks.

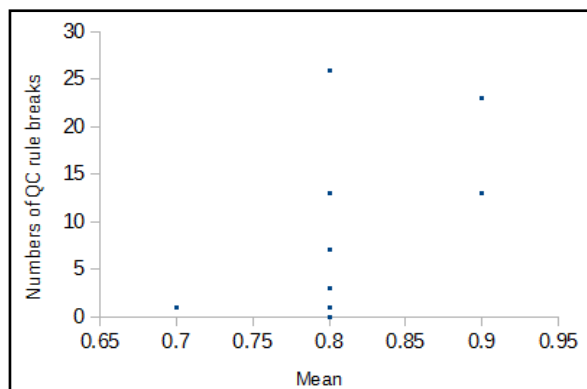


Figure 10: Direct Billirubin-Total QC Rule break for various mean observed

In above graph for DBIL, use of 0.2 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

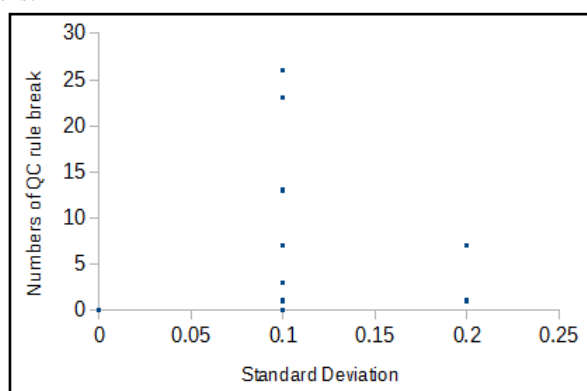


Figure 11: Direct Billirubin-Total QC Rule break for various SD observed

In above graph for DBIL, use of increasing unit as SD, results in decrease in number of QC rule breaks.

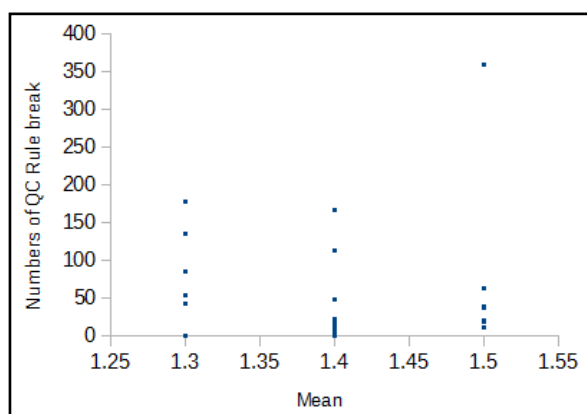


Figure 12: Creatinine-Total QC Rule break for various mean observed

In above graph for CR, use of 1.4 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks

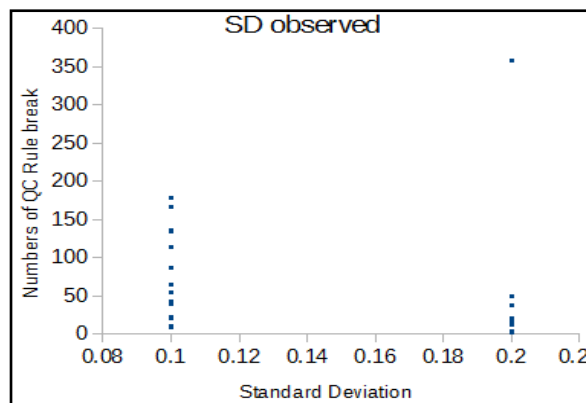


Figure 13: Creatinine-Total QC Rule break for various SD observed

In above graph for CR, use of 0.2 mg/dl as SD, shows less rule break than 0.1 mg/dl as SD

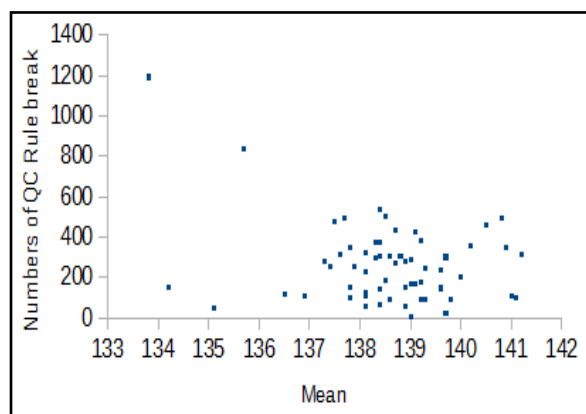


Figure 14: Sodium-Total QC Rule break for various mean observed

In above graph for Sodium, use of 140 IU/L as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

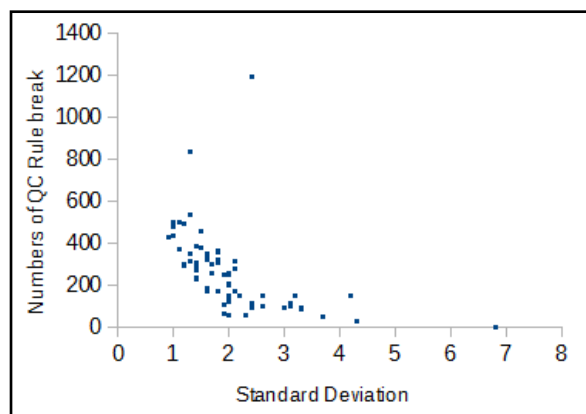


Figure 15: Sodium-Total QC Rule break for various SD observed

In above graph for Sodium, use of increasing unit as SD, results in decrease in number of QC rule breaks.

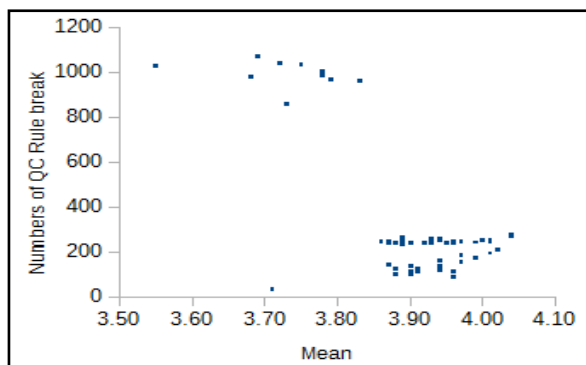


Figure 16: Potassium-Total QC Rule break for various mean observed

In above graph for Potassium, use of 3.90 IU/L as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

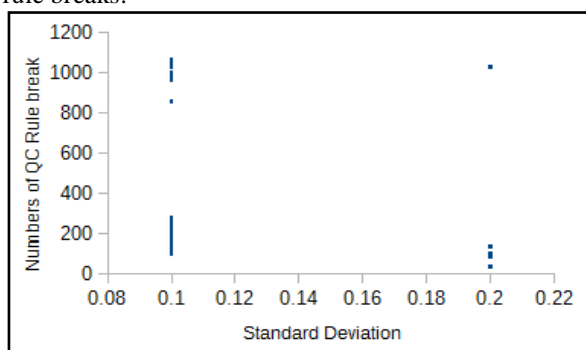


Figure 17: Potassium-Total QC Rule break for various SD observed

In above graph for Potassium, use of 0.2 IU/L as SD, shows less rule break than 0.1 IU/L as SD

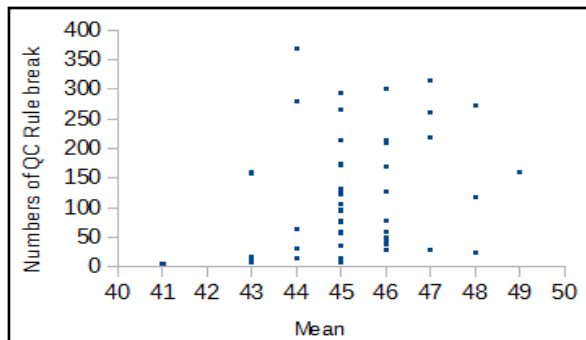


Figure 18: Urea-Total QC Rule break for various mean observed

In above graph for Urea, use of 41mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule break

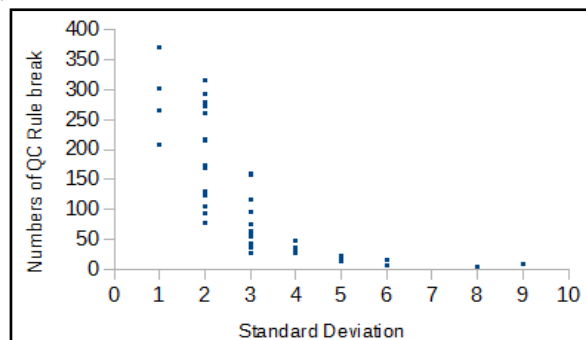


Figure 19: Urea-Total QC Rule break for various SD observed

In above graph for ALT, use of increasing unit as SD, results in decrease in number of QC rule breaks.

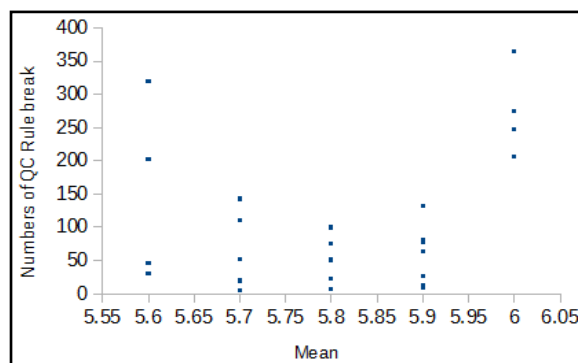


Figure 20: Total protein-Total QC Rule break for various mean observed

In above graph for Total Protein, use of 5.8 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

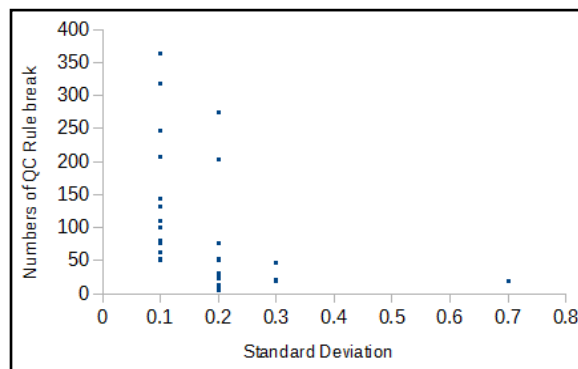


Figure 21: Total protein-Total QC Rule break for various SD observed

In above graph for Total Protein, use of 0.1 mg/dl, 0.2 mg/dl and 0.3 mg/dl as SD, results in decrease in number of QC rule breaks.

The illustration 16 to illustration 35 shows that there is relationship between number of QC rule breaks and SD as well as mean used for calculation.

Graphs of mean verses number of QC rule breaks shows certain “optimum” mean where the number of QC rules broken is minimum. On increasing and decreasing mean from “optimum”, the number of times QC rules broken, increases.

For example in Illustration 34 for Total Protein, use of 5.8 mg/dl as mean shows minimum number of QC rule breaks, while decreasing or increasing mean, results in increase in number of QC rule breaks.

Graphs of SD verses number of QC rule breaks shows that number of QC rules broken are less with increase in SD, as expected. For example in Illustration 35 for Total Protein, use of 0.1 mg/dl, 0.2 mg/dl and 0.3 mg/dl as SD, results in decrease in number of QC rule breaks.

The high range of variation in rejection rate based on 2(2S) and 1(3S) QC rules on use of different slots of 20 QC results observed in the study for various analytes are likely to be mainly due to following reasons

- Statistical variation in sample mean and SD from population mean and SD.
- Day to day variation in equipment performance.
- Day to day variation in reagent quality.
- Day to day variation in reconstitution of QC materials.
- Use of uncalibrated volumetric flask for reconstitution.
- Reconstitution with water not properly deionized.
- Inappropriate delivery of deionized water from volumetric flask into QC/calibrator bottle.
- Laboratory personnel performing reconstitution procedure may be suffering from vision problem, which may lead to erroneous reconstitution.
- Care not taken from light protection while performing reconstitution procedure, which may lead to degradation of bilirubin component of lyophilized powder.

4. Discussion

4.1 Statistical variation in sample mean and SD from population mean and SD

There is increasing uncertainty in measurement of SD and mean of a population when sample size is small. Use of 20 samples can result in variation in SD as much as 30% at 95% confidence. Thus, increasing sample size to 60-100 may result in more stable mean and SD, resulting in lesser false rejection and lesser false acceptance of run.

4.2 Day to day variation in equipment performance:

It is not necessary that the equipment performing various examinations, work with same efficiency all the time. It is subjected to various instrumental and technical error. For example,

- Failure in sampling system
- Failure in aspiration system of reagents
- Changes in analyzer's photometric unit / flow cell / measuring unit
- Electricity fluctuation leading to interruption in technical performance

4.3 Day to day variation in reagent quality:

Laboratory may use diagnostic kit provided by manufacturer or may use in-house reagents using valid method. In both the instances, there may occur variations. For example,

- Lot to lot variation in reagents.
- Change in Material used for reagent preparation
- Change in glassware used for reagent preparation
- Variation in quality of glassware cleaning
- Shift in calibration of volumetric glassware and pipettes
- Error in weighing of any ingredient while making reagent in case of in-house reagent usage.
- Change in calibration of weighing machine.

4.4 Day to day variation in reconstitution of QC materials:

Reconstitution of lyophilized QC and calibrator is subjected to inter individual as well as intra individual variation. For example,

As indicated in CLSI C24-A2 document, there is requirement of overlapping QC lots. Use of very high number of samples will result in longer overlapping period and greater cost to the laboratory. For laboratories, it may be difficult to procure large amount of same lot QC sera, thus, use of very high number of samples for measurement of mean and QC is impractical.

Thus, laboratories should use a compromise between too little sample size and impractically large sample size. While, 60-100 sample size may be adequate to prevent problem of variation in QC mean and SD, separate study is required to analyze the issue.

The laboratory can take several steps to decrease variation in QC rejection observed on taking mean and SD of results from different period. Instead of taking 20 reading in 10 days (twice a day), 20 readings may be taken from on reading per day for 20 days. This strategy may result in more stable mean and SD, due to inclusion of wider variation in factors affecting laboratory performance, at the same time not increasing the cost of QC materials and reagents used.

References

- [1] NCCLS. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition. NCCLS Document C24-A2 (ISBN1-56238-371-X). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, 19087 USA, 1999.
- [2] Westgard JO, Barry PL, Hunt MR. A Multi-Rule Shewhart Chart for Quality Control in Clinical Chemistry. *Clin. Chem.* 1981; 27(3):493-501.