Use of Beyond-Linearity Quality Control Sera for Routine Internal Quality Control of Glucose and Creatinine

Nilam Karbhari*, Dipti Karbhari, Shailesh Patel*****

*3rd Year Resident, *** Professor and Head, Department of Biochemistry, Government Medical College, Surat-395001, Gujarat, India

Abstracts: Introduction: While linearity of assay is tested during validation, there is no day to day check on changes thereof, although change in linearity will affect results around the linearity limits. Objectives: The study was performed to see if introduction of such beyond linearity QC program is useful to the laboratory or not. Methods: Serum control pool of beyond linearity range was prepared and analysed along with the routine quality controls and the incidences of rejections of routine internal controls and beyond linearity controls were compared. Results: There was general correlation in rejection incidences between routine QC and Beyond-linearity QC for both 1(3S) and 2(2S) QC rules. Interpretation and conclusion: The study shows that, there is no major increase in error detection rate on introduction of beyond-linearity QC. However, there was increase in warnings 1(2S) signs with beyond linearity QC. [Nilam K NJIRM 2017; 8(1): 77-81]

Key Words: Linearity, Quality Control, LJ Chart, Creatinine, Glucose.

Author for correspondence: Dr. Nilam Shivram Karbhari,156, Kalpananagar Society, Opposite Agriculture College, Vijalpore- 396450, Ta-Jalalpore, Dist.-Navsari M: 7490022677 E- Mail: neelamkarbhari31@gmail.com

Introduction: Internal Quality Control program and External Quality Assurance Program are backbone of quality system of any modern day clinical laboratory. A laboratory generally follows 2 level quality checks, normal and abnormal level. But quality check of beyond linearity range is not included. Their introduction in routine IQC program may be useful to detect analytical errors earlier than routine IQC program.

Beyond-linearity QC sera are generally not available in market. This study aims to look as possibility and suitability of beyond-linearity in-house QC sera in routine QC program of the laboratory. The study uses the in-house beyond-linearity QC to find incidences of QC rules broken; and whether it gives any additional information over and above routine QC procedures.

Control Material: Each control materials used should be able to provide information about what is going on with the testing process, material composition should be similar to or identical with patients sample matrix being analysed and should be homogeneous and stable at least for one year. There should be no or least vial to vial variability and control materials need to be different from the calibrator materials to ensure that the QC procedure provides an independent assessment of system performance[1].

Types of Control Material: Among the various types of materials used in quality-control programs are human serum, animal sera, human plasma "converted" into a serum-like state, and bovine albumin solutions[2]. Materials from human sources have generally been preferred, but because there is some risk of hepatitis infection, bovine and synthetic materials offer a certain advantage in safety.[1]

1.Liquid Stable Control Material: Advantage- Liquid control materials have the potential advantage of eliminating errors caused by reconstitution. Disadvantage- The matrices of these control materials may contain other materials which are a potential source of error with some analytical methods and instruments. Generally they are less stable.[1]

2. Lyophilized Control Material: This type of control materials are available in dry powder form that can be reconstituted by using deionized water or specific diluent. They are more stable. Error in reconstitution is a probable source of error in QC.[2, 3]

3. Frozen Serum Pool: A laboratory can use leftover patient serum pool as a control material. Various studies show that such frozen serum pools are better quality control specimens than lyophilized material. However, freezing may affect activity of certain Analyte in QC.

Clinical Decision Levels of control material: For most Analyte-method combinations, a minimum of two levels (concentrations) of control materials is recommended. Where possible, Analyte concentrations should be at clinically relevant levels to reflect values encountered in patient specimens. Concurrently using matrix control samples at different levels allows application of additional quality control rules which improve interpretation of analytical error

(i.e., proportional vs. constant, random vs. systematic).

To ascertain the acceptability of patient data, additional control materials may be added at clinical decision levels appropriate for the test and analytical system. Laboratories should plan their quality control strategies to include these important decision levels unless performance can be monitored with fewer levels.[4]

Certain manufacturing companies and their multilevel controls concentration values for Glucose and Creatinine are listed in table 1 and 2.

Table 1: Randox- Multilevel controls and concentration

Randox			
		Human assay (mg/dl) [5, 6]	**Bovine assay (mg/dl)[7, 8, 9]**
Level 1	Glucose	NA	65.4
	Creatinine	NA	0.91
Level 2	Glucose	110	115
	Creatinine	1.49	1.6
Level 3	Glucose	283	283
	Creatinine	4.09	5.71

Table 2: Bio-Rad- Multilevel controls and concentration

		BIORAD (mg/dl) [10]
Level 1	Glucose	57.7
	Creatinine	0.76
Level 2	Glucose	121
	Creatinine	1.94
Level 3	Glucose	362
	Creatinine	6.68

Above datashows that, generally commercial manufactures that provide control materials which do not involve beyond linearity range. So that range can be included in control program by using in house pool from patient's serum.

Methods: The study was performed in Clinical Biochemistry laboratory, New Civil Hospital, Surat for Glucose and Creatinine.

Linearity experiment: After performing the linearity experiments according to NCCLS document EP6-A [11], the linearity limit for glucose and creatinine were set as 503 mg/dl and 22 mg/dl respectively.

QC preparation: 15 bottles of normal level Calibrator sera from Randox, obtained in Lyophilized form were reconstituted with 5 ml deionized water using 5 ml TC volumetric flask calibrated gravimetrically by schimatzu weighing scale which is calibrated by sartorious loose weights(10mg, 100mg, 1gm) which are calibrated externally by NABL accredited calibration laboratory. Reconstituted sera allowed to dissolve properly for an hour and collected in 100ml bottle and mixed.

500mg of glucose powder, AR grade (Lobachimie lot-A189331605) and 23mg of creatinine powder, AR grade (Lobachimie lot-SL47131206) are weighted through schimatzu weighing scale and added in QC pool and waited to dissolve. Prepared pool was filtered using Whatman filter paper and 0.2 ml aliquoted in to 1.5 ml cups, sealed with paraffin film and kept at -20'C.

Obtaining SD and Mean: Each beyond linearity QC cup was allowed to defrost, thawed, mixed by inversion and analysed three times a day for 20 days. Total number of QC data available for obtaining target and SD was approximately 60 for each Analyte.

Performing Daily QC: The obtained mean and SD were set and beyond linearity QCs were analysed along with routine IQC for next 60 days and were exported to LIS. Incidences and discrepancy of rule break of 1(3S) and 2(2S) rules between beyond linearity QC and routine IQC were noted.

Data analysis: The QC results of beyond-linearity QC and routine QC samples were exported from LIS to spreadsheet and rejections were calculated. Average rejection rate and discrepancy in rejection rate were calculated.

Result: Table 3 shows obtained target value and SD for Glucose and Creatinine from 20 days analysis.

Table 3: Target and SD for Glucose and Creatinine obtained from 60 data points

	Glucose (mg/dl)	Creatinine (mg/dl)
Target	755	25.7
SD	18	2

Using these target value and SD beyond linearity controls were analysed along with routine controls for next 60 days (157 no. of observations). Both analytes control results were plotted in charts to find any out of control result i.e., 2(2S) and 1(3S) rule break. Results for Glucose and creatinine in routine and beyond-linearity controls are shown below.

Table 4 and 5 shows observation and its results of all 3 levels controls analysed at same time for both analytes.

Table 4: Data Analysis- Glucose

Glucose			
	Normal range	**Abnormal range**	**Beyond linearity range**
Target value	114	281	755
No. of observation	157	157	157
Average	113	280	742
SD	3	6	24
CV	2.41	2.06	3.23

Table 5: Data Analysis- Creatinine

Creatinine			
	Normal range	**Abnormal range**	**Beyond linearity range**
Target	1.4	3.9	25.7
No. of observati	157	157	157
Average	1.4	4	26
SD	0.2	0.3	2.2
CV	9.59	5.46	8.34

As in graph shown, as time passed, the value of Glucose in QC samples gradually decreased (illustration3 and 4). So new target value was assigned from new results. And using this target and SD remaining QC were analysed. Following graph shows results for Glucose QC for all three ranges.(illustration1,2,3,4)

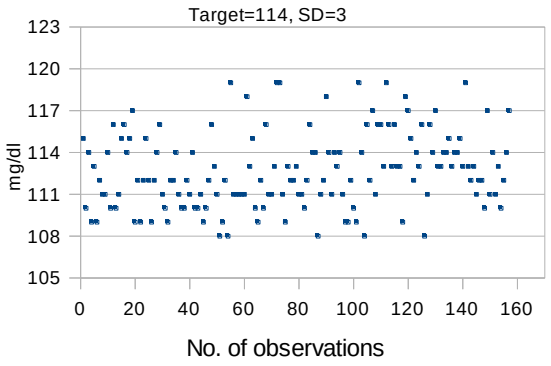

Illustration 1: Glucose control chart- Normal range

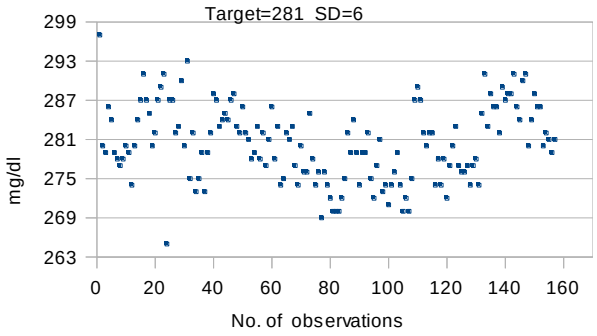

Illustration 2: Glucose control chart- Abnormal level

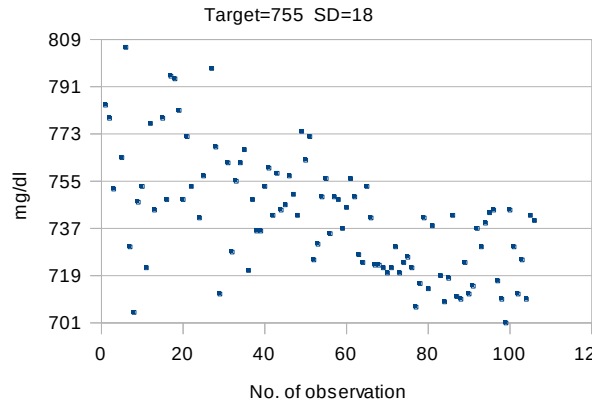

Illustration 3: Glucose control chart- Beyond linearity range (part-1)

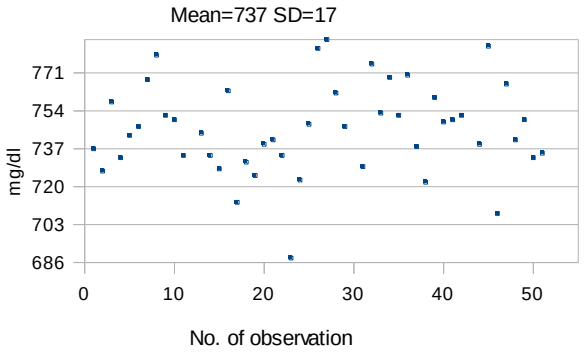

Illustration 4: Glucose control chart- beyond linearity (part-2)

Following graph shows results for Creatinine QC for all three ranges.(illustration5,6,7)

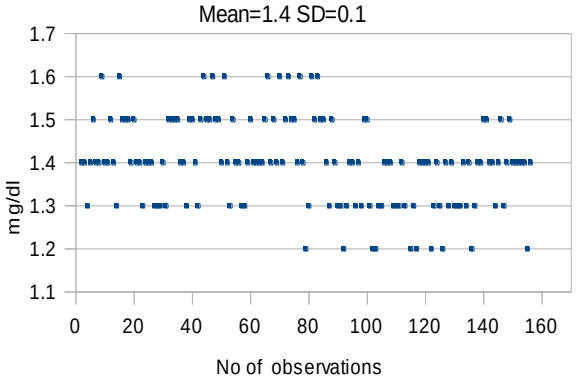

Illustration 5: Creatinine control chart- normal range

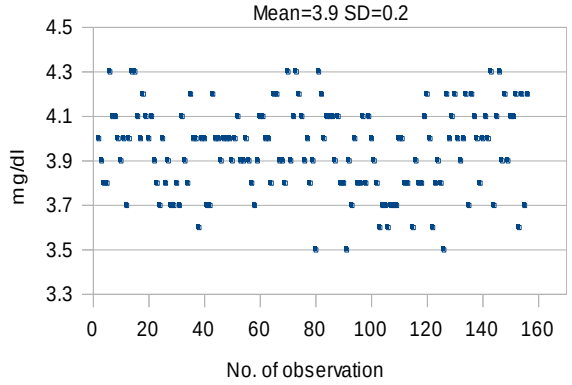

Illustration 6: Creatinine control chart- Abnormal range

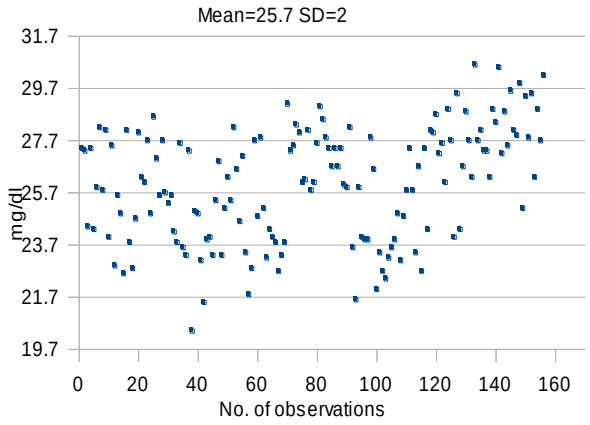

Illustration 7: Creatinine control chart- Beyond Linearity

Incidences of rule break in all controls are described in table 6 and 7.

Table 6: Incidence of rejection for Glucose

Level Of QC	Incidence of rejection for Glucose		
	1(3S)	2(2S)	1(2S) (Warning)
Routine-Normal range	0	0	0
Routine-Abnormal range	0	0	2
Beyond linearity range	3	0	35

Table 7: Incidence of rejection for Creatinine

Level Of QC	Incidence of rejection for creatinine		
	1(3S)	2(2S)	1(2S) (Warning)
Routine-Normal range	1	0	2
Routine-Abnormal range	1	0	2
Beyond linearity range	0	0	7

Discussion: The tables above show that, there is general correlation in rejection incidences between routine QC and Beyond-linearity QC for both 1(3S) and 2(2S) QC rules. However, the study surprisingly found higher incidences of 1(2S) rule break, which is not considered for rejection; instead it is considered a warning rule. Higher incidence of warning rule break indicate that there are many incidences in a laboratory where performance of laboratory deteriorates but conventional QC levels do not detect them, but the beyond-linearity QC detect them. Some such situations, where on beyond-linearity results will be affected, without affecting results within lower ranges are deterioration in critical component of reagent system. Such situation may arise due to long on-board stay of reagent, low on-board stability of reagents and unfavourable room temperatures and contamination from other reagents due to reagent probe carry over. Whether attention to such increased 1(2S) warnings will improve laboratory performance or not, and whether it is a false alarm needs to be studied.

The study shows that 1(2S) warnings are relatively high in Glucose as compared to Creatinine assay with beyond-linearity QC. Glucose measurement is based

on Glucose Oxidase and peroxidase enzyme. Such enzyme based reagents are more unstable as compared to non-enzymatic chemical reactions like Jaffe's. Decreased on-board reagent volumes and frequent refilling of on-board reagents from on-shelf stock, improvement in on-board reagent temperature may help decrease such warnings. The study showed relatively less number of QC rule violation. Increase in number of readings, by extending the study over more than a year would help arrive at more reliable

Conclusion: Use of beyond-linearity QC is not a frequently done research. The study analysed the use of such material in routine QC practices. The study shows that, there is no major increase in error detection rate on introduction of beyond-linearity QC. However, such QC gives greater number of warning signs, Watchful observation and correction of such warning may improve laboratory quality by signifying early sign of impending system failure. Reagents are major component of determinants of reagent linearity. Improvement in reagent housekeeping may help decrease beyond-linearity QC rule violation and help laboratory improve quality.

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Conflict of interest: None
Funding: None
Cite this Article as: Nilam K, Dipti K, Shailesh P Use of beyond-linearity quality control sera for routine internal quality control of Glucose and Creatinine. Natl J Integr Res Med 2017; 8(1):77-81