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Adopting Traditional Quality Control Protocols to Monitor Molecular Diagnostic Tests

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Abstract

Quality Control (QC) for molecular diagnostic tests encounters many challenges. The traditional QC practice of using homogeneous (consistent lot to lot) QC materials could be useful for molecular diagnostics but current QC practices for genetic tests are limited in their ability to detect and prevent errors. To address the QC gaps in molecular diagnostic quality assurance, protocols can be adopted from routine clinical chemistry laboratories. The objective of our study was emerged to find out the effectiveness of adopting the routine and homogeneous control samples to be monitored and statistically analyzed for shifts and trends to detect potential test performance problems before failure occurs and even before the establishments of actual mean and standard deviation. We used 10 curves of different Molecular Diagnostic Tests comparing the failure occurred before and after establishments of actual mean and standard deviation as an example of adoption. The result was highly insignificant ($p > 0.05$) comparing the number of finding before and after establishments of actual mean and standard deviation as an example of adoption. We concluded that adoption of Molecular Diagnostic Tests quality control charts could be an available and good solution to overcome the challenges facing current QC practices for genetic tests.

Introduction

Molecular diagnostic tests Quality Control (QC) encounters many challenges and we consider the cost effectiveness challenge as priority followed by new and rapidly evolving technologies, high expectations of accuracy for once-in-a-lifetime genetic tests, lack of quality control materials, lack of standardized calibrators, lack of quantitative test system outputs and software to track them, and the almost daily appearance of new test targets. In the face of such issues, clinical laboratories struggle to develop appropriate quality assurance programs for the molecular diagnostic tests they conduct (1).

The accuracy of molecular testing is currently questionable, but concerns about quality in molecular diagnostic tests can only be quantified and effectively addressed with reliable data availability. Although monitoring molecular diagnostic test system outputs and applying statistical analysis provide a good way to obtain data on accuracy and precision, such traditional QC strategies which have been used routinely in other laboratory disciplines have been slow to take root in molecular diagnostics. (2)

The traditional QC practice of using homogeneous (consistent lot to lot) QC materials could be useful for molecular diagnostics in identifying degrading or defective system components before an actual test failure. Faced with the limited commercial availability of QC materials, some labs pool patient samples to create a reproducible source of such materials. Furthermore, new molecular systems have quantitative outputs, such as fluorescence (signal strength) or allelic ratio, which can be tracked to monitor test system performance. Statistical analysis of the QC results over time can establish expected variations. Such results can then be serially plotted on Levey-Jennings charts to monitor the test system for shifts or trends in performance. “Westgard Rules” can be applied to determine when corrective action should be taken to prevent test failure same way like routine biochemistry diagnostic laboratories (traditional QC practices). (3)

These traditional QC practices satisfy best practice and CLIA regulations. Particularly pertinent to QC practices but often not stringently followed by molecular labs are CLIA Sections. The laboratory must establish and follow written QC procedures for monitoring and evaluating the quality of the analytical testing process of each method to assure the accuracy and reliability of patient test results and reports and For each test system, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytical process, and The control procedures must Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance. Monitor over time the accuracy and precision of test performance. As in other laboratory disciplines, molecular QC should monitor all test

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2023.4.inj59
Vol. 27 Iss. 4 (2023)

components including the extraction step and the material should be homogeneous so that shifts and trends of QC results can be assumed to be due to the test system, not the QC material. (4)

Molecular test systems are now more complex and expensive. Since samples for genetic molecular tests cost at least $40 each. The traditional QC practice of using homogeneous QC materials could be useful for molecular diagnostics in identifying degrading or defective system components before an actual test failure. Faced with the limited commercial availability of QC materials, Furthermore, new molecular systems have quantitative outputs, such as fluorescence (signal strength) or allelic ratio, which can be tracked to reflect test system performance. Statistical analysis of the QC results over time can establish expected variations. Such results can then be serially plotted on Levy-Jennings charts to monitor the test system for shifts or trends. Westgard rules can also be applied to determine when a corrective action should be taken to prevent test failure. (5)

If we consider the retesting is a significant problem beside the above mentioned challenges and the low numbers of tests that could be processed within the same lot number, and we stressed on the above mentioned necessity of applying QC results within routine Levy-Jennings charts and quality rules, the emerge of adopting this rules get on the surface of molecular laboratories.

In addition, Current QC practices for genetic tests are limited in their ability to detect and prevent errors. Tests for rare mutations may never be subject to quality assessment, therefore an error prevention strategy or even an estimate of error rates for detecting those alleles is not possible. For more common alleles in which quality controls are routinely run, plotting and analyzing the data for error prediction is not usually practiced. Also, rotating 2-3 controls per run does not generate sufficient data about the accuracy of detecting genotypes not included in the controls. The traditional quality resources and practices used in clinical chemistry laboratories compared with those used for molecular diagnostic tests are unbalanced with most of them not fit for the molecular diagnostic tests (6).

To address the QC gaps in molecular diagnostic quality assurance, protocols can be adopted from routine clinical chemistry laboratories. Traditionally, the quantitative data generated from testing homogeneous control samples is monitored and statistically analyzed for shifts and trends to detect potential test performance problems before failure occurs and even before the establishments of actual mean and standard deviation. With the exception of high volume virology testing, this approach is new to molecular diagnostics laboratories. However, thought leaders and early adopters of molecular testing are beginning to use such protocols. (7)

In an attempt to overcome defaults of many controls like that are made of synthetic materials diluted in matrices unrelated to patient specimens (although whole organisms may be available). Moreover, in-kit controls are optimized to function with specific assay platforms and reagents. Indeed, assay reagents are developed and tested by assay manufacturers with the same materials making up the controls provided in their kits, hence introducing a potential prejudicial bias. Additionally, when assays also include standards, the in-kit control(s) typically derive from those standards. Therefore, if an issue impacts the standards, the in-kit controls are affected in the very same manner, making everything appear as normal, while the underlying issue may continue to impact patient results. Hence, it is critical to use well designed, independent, and unbiased third-party QC controls in addition to (or instead of) the in-kit controls. Such independent molecular controls are designed to mimic clinical specimens and consist of whole inactivated pathogens in relevant clinical matrices, so they are affected by the entire analytical process in the exact same manner as the same pathogens found in the patient specimens. Hence, unlike with synthetic controls, each of the extraction, amplification and detection steps is fully controlled in an unbiased and thorough manner. A laboratory in a cutting-edge field like molecular infectious-disease testing merits an exceptional quality control program to strengthen confidence in laboratory performance and support optimal patient care (8)

Materials and methods:

10 curves of different Molecular Diagnostic Tests were used comparing the number of failure (according to traditional “Westgard Rules”) occurred before and after establishments of actual mean and standard deviation as an example of adoption. Statistical analyses were performed by standard methods, and a “p” value of less than 0.05 is considered statistically significant.

Results:

Charts for QC results plotted on Levey-Jennings charts to monitor according to “Westgard Rules” number of failure occurred before and after establishments of actual mean and standard deviation as an example of adoption (on different number of QC processing):

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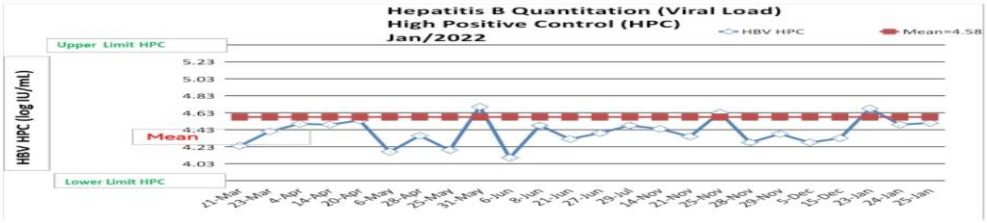

Chart (1a): Manufacturer's Mean Control Lot #511744 (Exp. Date: (11-FEB-2022

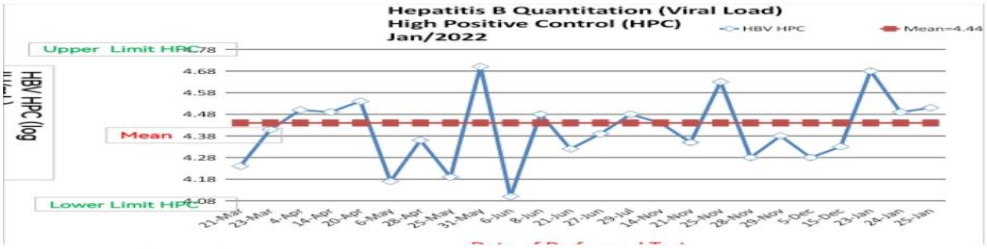

Chart (1b): 5 points mean

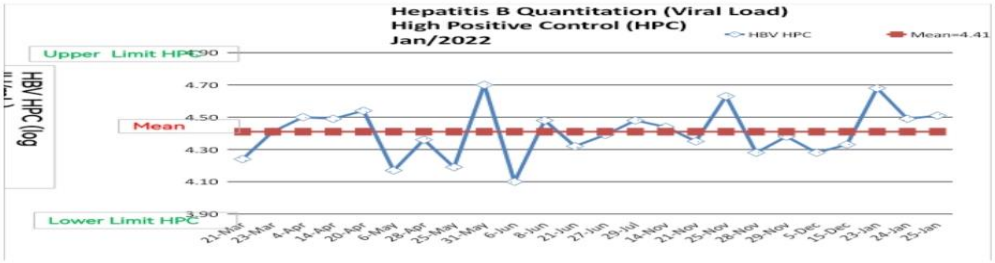

Chart (1c): 10 points mean

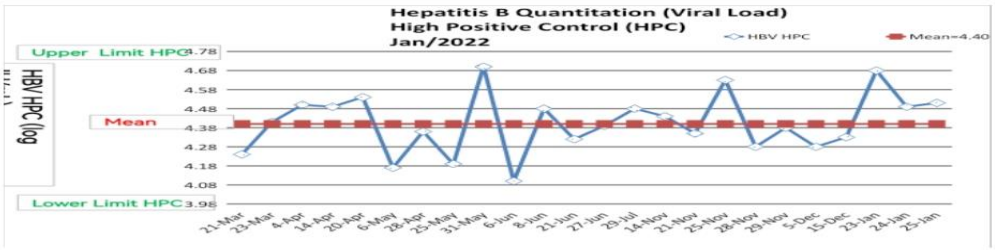

Chart (1d): 15 points mean

DOI: 10.5123/inj.2023.4.inj59
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

INJ INTERNATIONAL NEUROUROLOGY JOURNAL

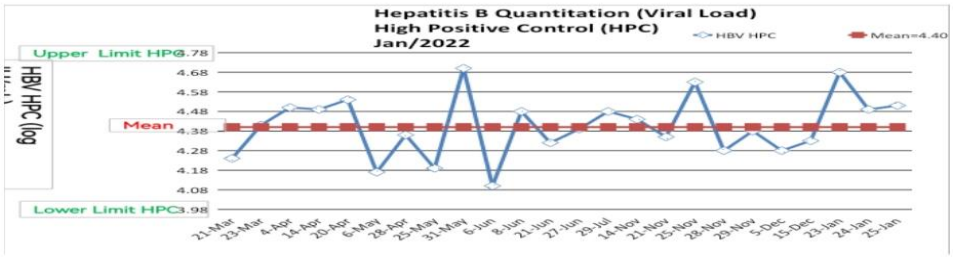

Chart (1e): 20 points mean

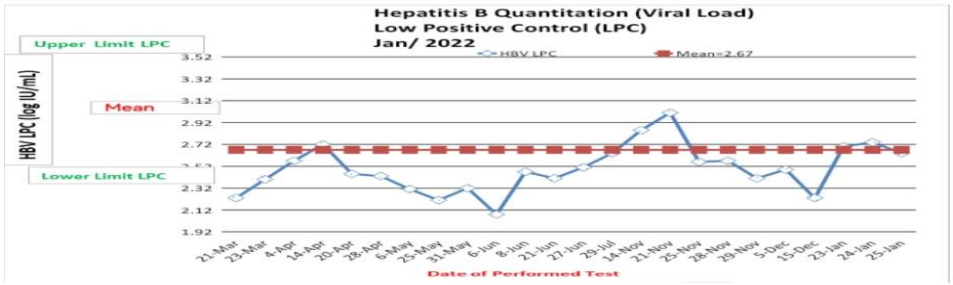

Chart (2a): Manufacturer's Mean Control Lot #511744 (Exp. Date: (11-FEB-2022

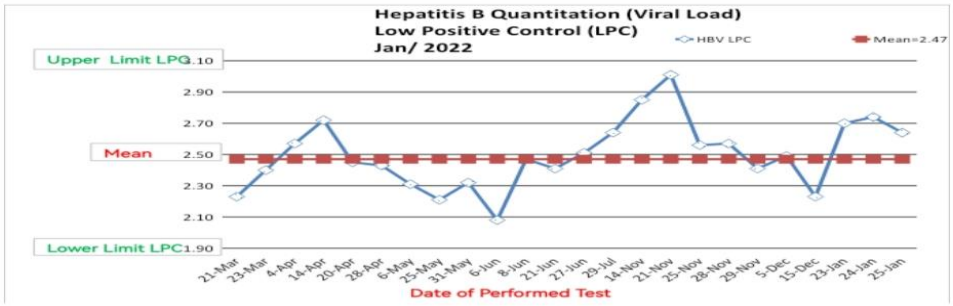

Chart (2b): 5 points mean

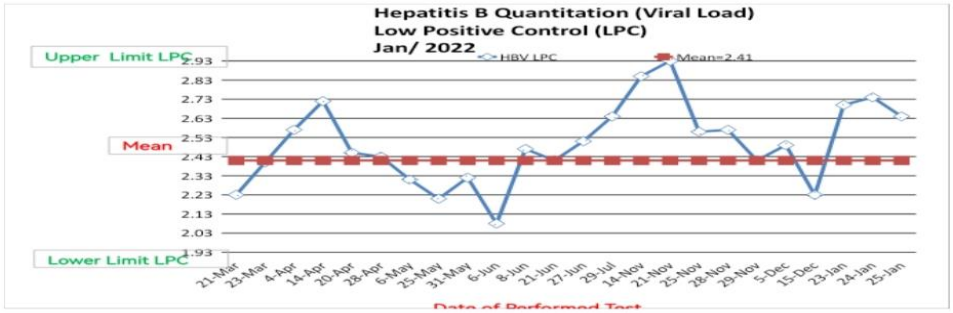

Chart (2c): 10 points mean

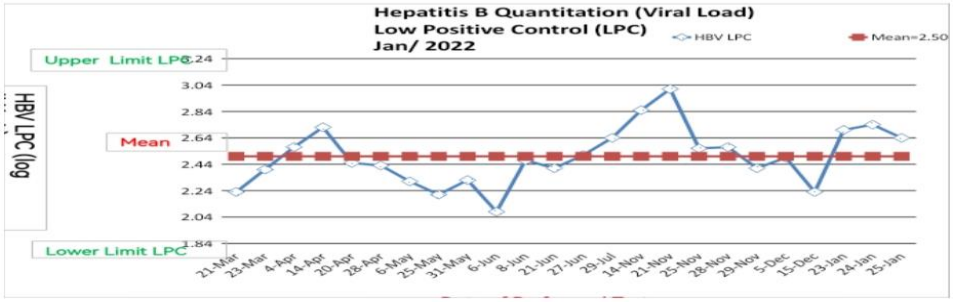

Chart (2d): 15 points mean

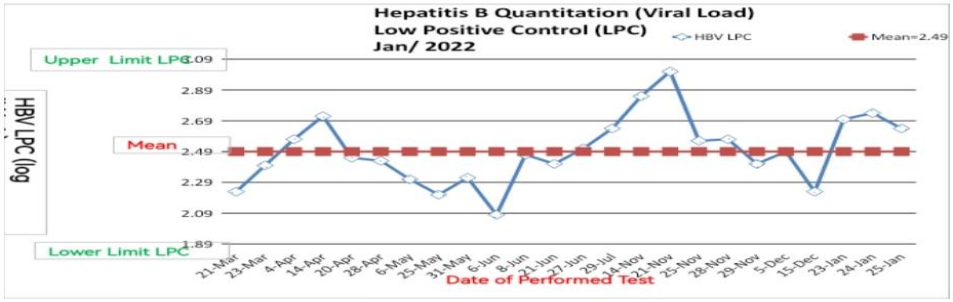

Chart (2e): 20 points mean

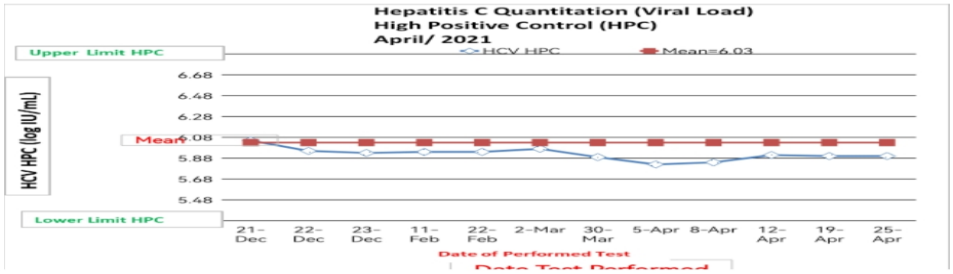

Chart (3a): Manufacturer's Mean Control Lot #501969 (Exp. Date: (29-Apr-2021

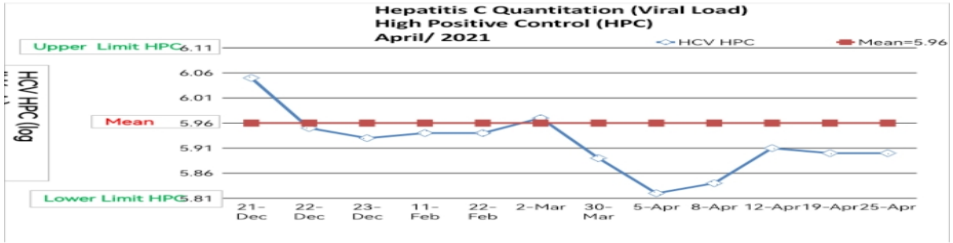

Chart (3b): 5 points mean

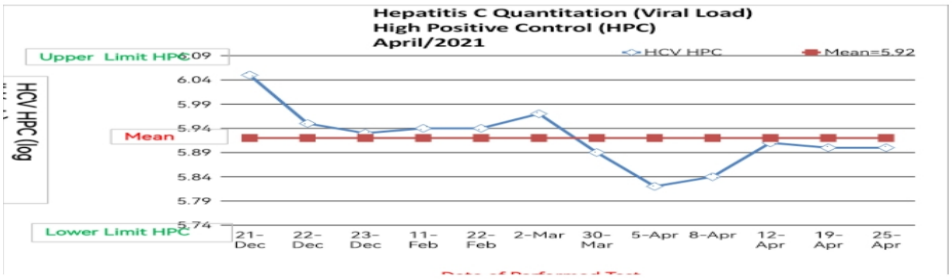

Chart (3c): 10 points mean

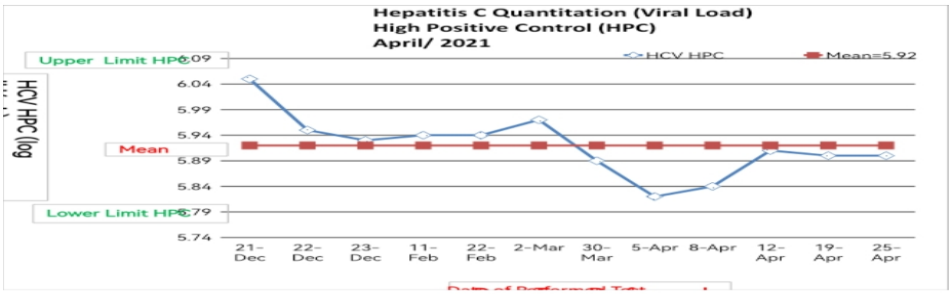

Chart (3d): 12 points mean

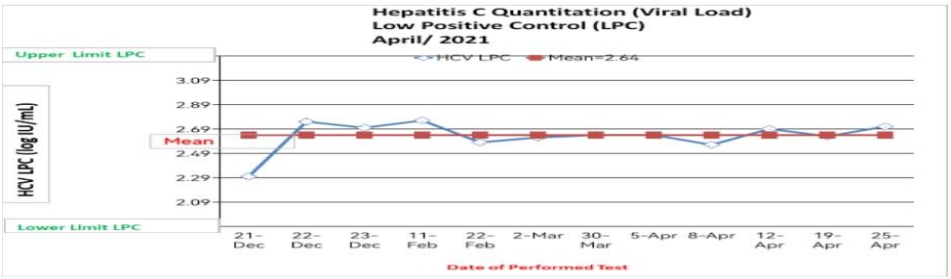

Chart (4a): Manufacturer's Mean Control Lot #501969 (Exp. Date: (29-Apr-2021

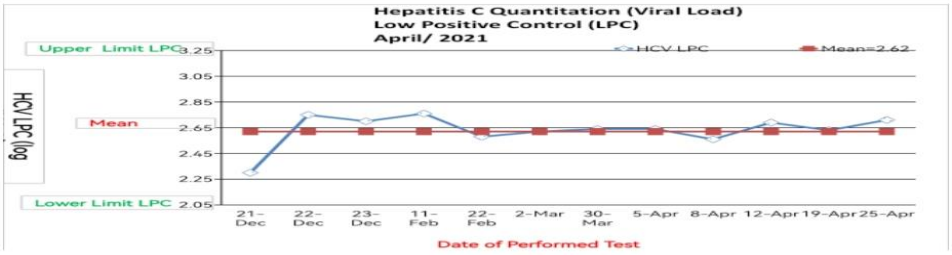

Chart (4b): 5 points mean

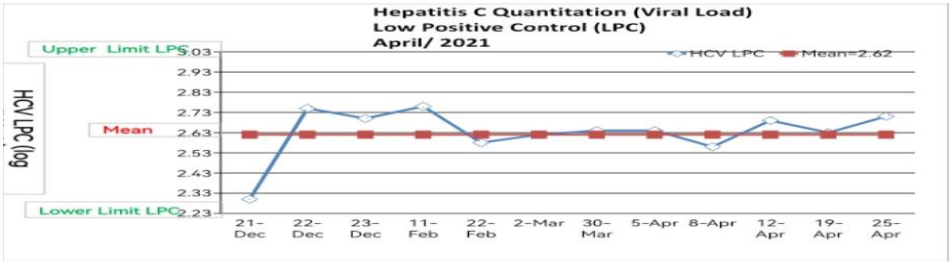

Chart (4c): 10 points mean

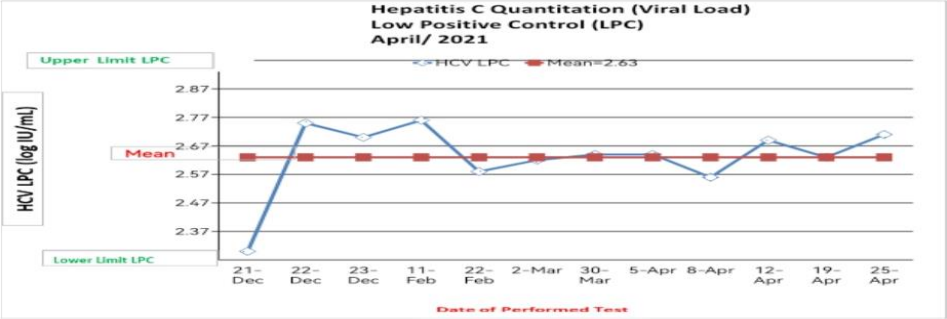

Chart (4d): 12 points mean

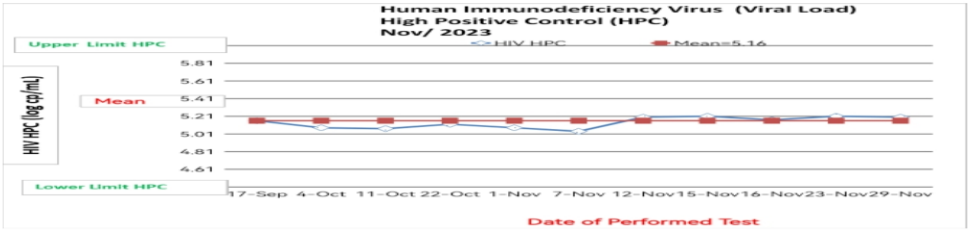

Chart (5a): Manufacturer's Mean Control Lot #381838(Exp. Date: (08-May-2024

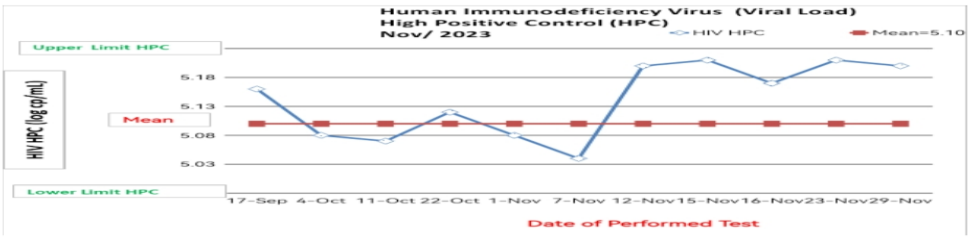

Chart (5b): 5 points mean

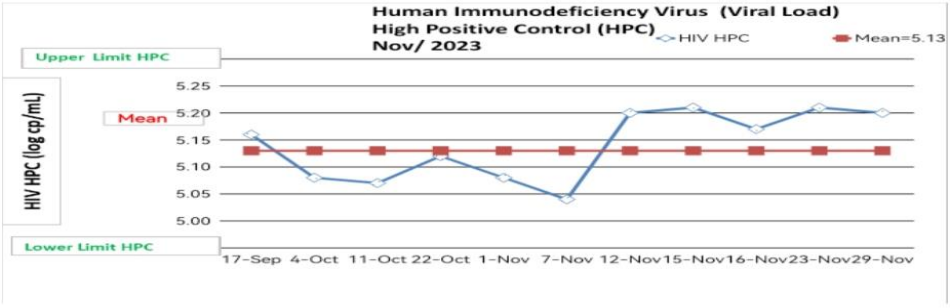

Chart (5c): 10 points mean

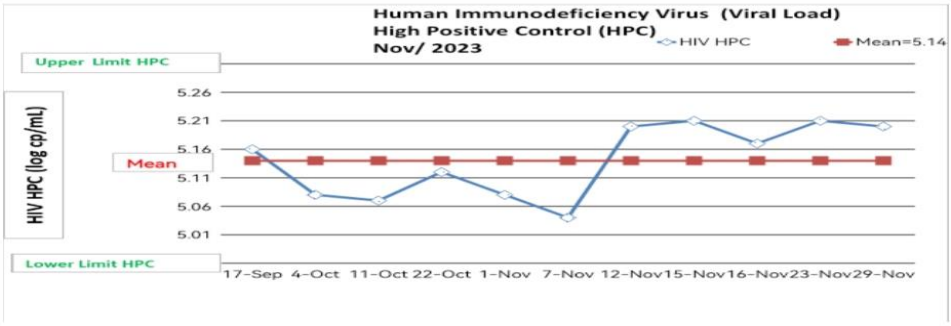

Chart (5d): 11 points mean

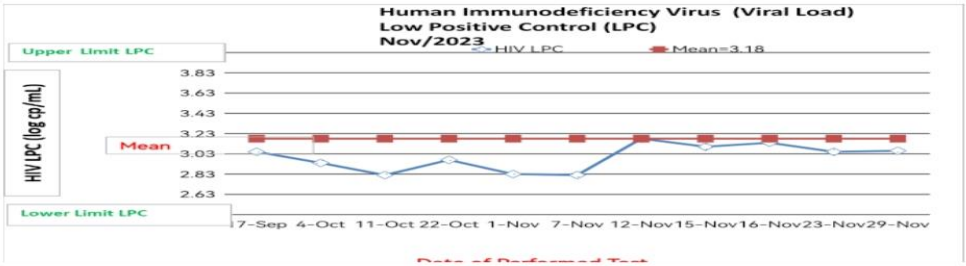

Chart (6a): Manufacturer's Mean Control Lot #381838(Exp. Date: (08-May-2024

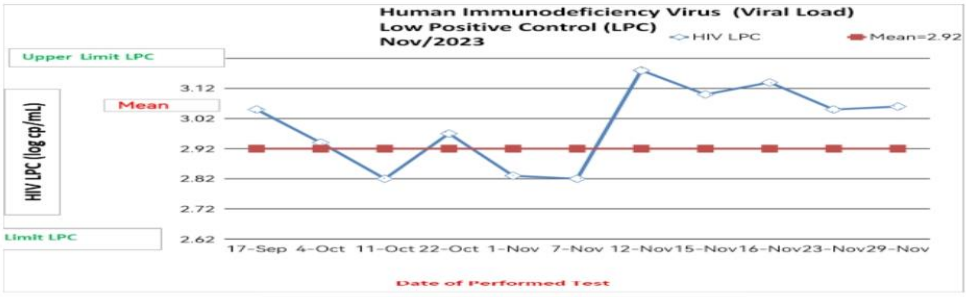

Chart (6b): 5points Mean

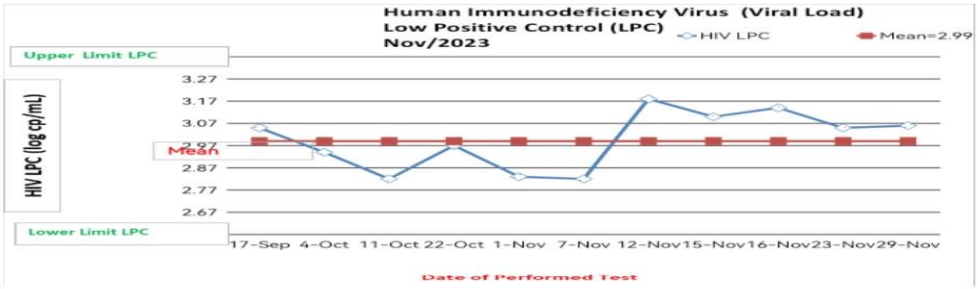

Chart (6c): 10 points Mean

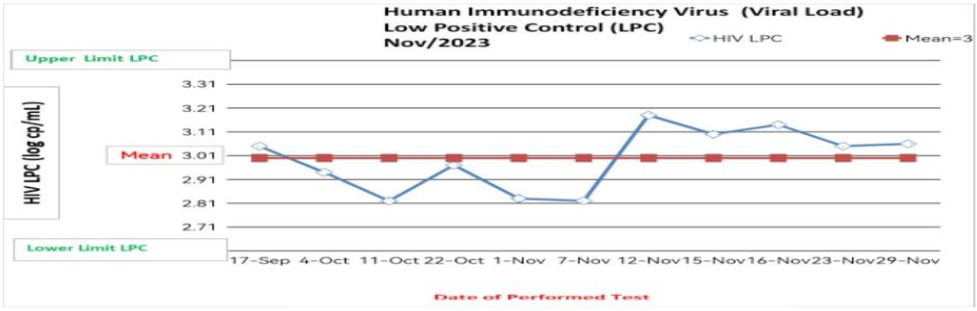

Chart (6d): 11 points Mean

Group No	Manufacturer's Mean & SD BEFORE establishments	AFTER establishments with 5 Points QC	AFTER establishments with 10 Points QC	AFTER establishments with 11-15 Points QC	AFTER establishments with 20 Points QC
1	0	1	1	1	1
2	1	0	1	1	NA
3	1	1	1	1	NA
4	0	0	1	1	NA
5	0	2	0	0	NA
6	1	1	0	0	NA
Total	3	4	4	4	NA

Table 1: Number of failure (according to traditional "Westgard Ru4les")

Total Number of failure BEFORE establishments	Total Number of failure AFTER establishments	P Value
3	4	0.1393

Table 2: p Value

Discussion

In general, it is attempted to reduce the cost of QC practices for genetic tests and to overcome the challenges facing.

The importance to define adopting and modified method to overcome that challenges are very important and one of many search target.

Our results showed a no significance difference between the number of failure (according to traditional "Westgard Rules") occurred before and after establishments of actual mean and standard deviation as an example of adoption in Molecular Diagnostic Tests QC Charts as p Value was.

Also there was no difference between the duplicate charts reflex on patient results.
The result of this study resolve the debate of using the routine and strict QC rules before or after completion of special numbers of QC material point in Levy-Jennings charts and quality rules could be manipulated according to laboratory circumstances and challenges or not.

From this point our result enforced the suggestion that Adoption of Molecular Diagnostic Tests quality control is a significance solution to overcome the challenges facing current QC practices for genetic tests.

Conclusion:

Adoption of Molecular Diagnostic Tests quality control charts could be an available and good solution to overcome the challenges facing current QC practices for genetic tests as there was no significant difference between the number of failure (according to traditional "Westgard Rules") occurred before and after establishments of actual mean and standard deviation as an example of adoption.

References:

1. James O. Westgard, PhD,Hassan Bayat PhD (CLS), and Sten Westgard, MS, (2023), Advanced QC Strategies, 1st Edition, Risk-Based Design for Medical Laboratories, 264 pages, 9" by 6" paperback, Library of Congress Control Number: 2022942184
2. James O. Westgard, PhD, (2023), http://westgard.org/index.pl?op=show;isa=Program;iid=93466, Extras for the Basic Quality Management Systems manual.
3. Secretary's Advisory Committee on Genetics, Health, and Society (SACGHS), "U.S. System of Oversight of Genetic Testing: A Response to the Charge of the Secretary of Health and Human Services," April 2008; available from Internet: http://www4.od.nih.gov/oba/sacghs/reports/SACGHS_oversight_report.pdf
4. S Liang et al., "Application of Traditional Clinical Pathology Quality Control Techniques to Molecular Pathology," Journal of Molecular Diagnostics 10, no. 2 (2008): 142-146.
5. Parviz Ahmed – Najed, Tester Ashavaid, Aldo Vacaflores Salinas, Jim Huggett, Kathryn Harris, Mark W. Linder, Katarina Baluchova, Werner Steimer, Deborah A. Payne, (2021), Current and future challenges in quality assurance in molecular diagnostics, Clinical Chimica Acta**,**Volume 519, August 2021, Pages 239-246
6. JO Westgard, Basic QC Practices, [ed. 3rd edition]. (Madison, WI: Westgard QC Inc., [ed. 2010]).
7. Marco Cascella; Michael Rajnik; Abdul Aleem; Scott C. Dulebohn; Raffaela Di Napoli., 2023 Features, Evaluation, and Treatment of Coronavirus (COVID-19) [Updated 2023 Jan 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554776/
8. David Gauthier, MA, Andy Quintenz, 2021, Quality control in the evolving landscape of molecular testing, https://www.mlo-online.com/management/qa-qc/article/21210718/quality-control-in-the-evolving-landscape-of-molecular-testing