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ORIGINAL ARTICLE

Interpretation of External Quality Assurance: How to and How Not to

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ABSTRACT

Precision and accuracy are the two pillars of quality in analytical testing process of a clinical laboratory. External quality assurance (EQA) holds a major share in shaping the analytical quality from accuracy perspective. But this depends on how laboratory perceives an EQA, understands it, and uses it effectively for inaccuracy assessment. External quality assurance has its own advantages and limitations, including the commutability of EQA sample, traceability of methods of comparison, the statistical procedure used for evaluation, etc. Our study discusses three brief case reports through which we have tried to explore the advantages and limitations of EQA.

Keywords: Clinical biochemistry, External quality assurance, Quality control, Traceability, Trouble shooting.

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INTRODUCTION

Clinical laboratory is a court of justice and laboratorians are advocates of quality. Quality management system (QMS) of a laboratory is its judiciary system. Laboratorians and advocates work in similar ways and move toward the same goal, customer safety. Both of them make a continual stride toward pinning in and eliminating out the endangered threats to the customer safety. In a clinical laboratory, errors produce a constant threat to patients' safety.[1] These laboratory errors are omnipresent in all phases of total testing process. Hence, we need a "round-a-clock" stringent vigilant system, in the form of a QMS to identify, investigate, and eliminate these errors and ensure patients' safety.

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One among the many potential errors in clinical laboratory is the analytical errors, which are bound to occur in the examination phase of testing process. The two most significant errors in the analytical process are imprecision and inaccuracy.[2] The clinical laboratories worldwide have developed two effective tools for the imprecision and inaccurate investigation; those being internal quality control (IQC) tool for imprecision monitoring and EQA tool for inaccuracy assessment.[3] In shaping the analytical quality from accuracy perspective, EQA holds a major share. But this depends on how a laboratory perceives an EQA, understands it, and uses it efficiently for inaccuracy assessment. General perception in clinical laboratory practice is that "an unacceptable EQA result means poor accuracy and otherwise."[4] But whether this perception holds true in all occasions and what are the factors to be considered while interpreting an EQA forms the focus of this study. Our case study includes three brief case reports through which we have tried to understand how to interpret and troubleshoot EQA.

MATERIALS AND METHODS

We did this study at the Division of Clinical Biochemistry, Department of Laboratory Medicine, MIOT Hospitals, Chennai, Tamil Nadu, India. Our laboratory participated in EQA program organized by the International Organization for Standardization (ISO) 17043:2010 accredited EQA provider (Bio-Rad). We included analysis of EQA results of three analytes, including serum immunoglobulin G (IgG) (Vitros 5600, immunoturbidimetric), creatinine kinase-MB (CK-MB) activity (Vitros 5600, immunoturbidimetric inhibition), and copper (Vitros 5600, 3,5-Di-Br-PAESA 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-(3-sulphopropyl) aniline).

RESULTS AND DISCUSSION

Case 1

On January 9, 2017, our laboratory encountered an analytical threat in the form of serum IgG "outlier" in EQA (Bio-Rad serum proteins program). Serum IgG EQA sample was processed in Vitros 5600 (immunoturbidimetric).[5] For serum IgG, EQA showed a Z score of +3.25, against the peer group comparison.[6] Our laboratory conducted a detailed investigation to understand the IgG EQA threat.

We followed a structured approach to the investigation based on the "Flow Chart for handling deviating EQA results" developed by External quality Control for Assays and Tests (ECAT) Foundation in the Netherlands[7] (Table 1). Six important aspects of investigation included evaluation of transcriptional error, presurvey issues, sample receipt/handling errors, test performance errors, data handling (by the EQA provider) errors, and errors in interpretation of EQA result. At the end of the investigation, we ruled out all possible causes of an "outlier" except the "test performance error." The following were the observations made with respect to "test performance":

- Internal quality control during the period of EQA sample processing was within the control limits established by the laboratory, though the Level 1 IQC, which was in measurement range of EQA result (Bio-Rad Immunology plus IQC), was constantly reported on higher side of the laboratory mean but within 2 standard deviation (SD) limits. The laboratory mean and SD limits were established as per the policy adopted by the division, which included minimum of 20 data points. The lab mean was 687 mg/dL and the lab SD was 37 mg/dL, while the manufacturer's mean and SD limits were 620 and 55 mg/dL. It was ensured that this positive deviation of the laboratory mean from the manufacturer's mean was not contributed by an imprecise calibration of IgG.
- With respect to measurand calibration, the laboratory had performed calibration 10 days prior EQA sample processing as per manufacturer's recommendations.[5] According to the manufacturer, the calibration showed a "Passed" status and was deemed to be successful.
- Taking into consideration the previous EQA results for IgG, no statistically significant "outliers" were reported except for the current one. Careful observation of the EQA results showed that an acceptable performance was displayed with previous EQA samples with values falling on the lower and middle range of the analytical measurement range (AMR), while the present "outlier" fell on the higher side of AMR for serum IgG. Hence, a high index of diagnostic suspicion of a "proportionate systematic error"

Table 1: Deviating EQA results

Types of errors		*Observation*	*Case 1*	*Case 2*	*Case 3*
Transcription error	a	Error in coding test results from the instrument	NA	NA	NA
	b	Error in reporting test results to EQA organization			
	c	Mixing up test results			
	d	Report results with wrong units			
	e	Report the wrong method and/or equipment			
	f	Error in data entry by EQA provider			
Presurvey issues	a	The EQA provider distributed by accident an inappropriate sample	NA	NA	NA
	b	Error in sample labeling			
	c	Error in packaging the samples			
	d	Error in distribution of samples			
	e	Problem with sample stability			
	g	Problem with sample homogeneity			
	h	Error in the instruction letter of EQA provider			
Sample receipt/ handling	a	Problems with the receipt of samples	NA	NA	NA
	b	Inappropriate storage of samples till use			
	c	Problems with reconstitution of samples			
	d	The instructions were not followed properly by the participant			
Test performance	a	Change in the instructions of manufacturer	NA	NA	NA
	b	Was there a problem with the equipment			
	c	Was there a problem with the reagents			
	d	Was there a problem with the IQC samples			
	e	Was there a problem with the test performance	Applicable		
Data handling EQA provider	a	The statistical procedure used is probably not appropriate for the distribution of the test	NA	NA	NA
	b	Error in establishment of the AV		Applicable	Applicable
	c	Error in presentation of results		NA	NA
Report and interpretation	a	Deviation in accordance with previous EQA results	NA	NA	NA
	b	Large variation in EQA results for the method used			
	c	Deviation is systematic for all EQA samples			
	d	Repeated analysis showed similar deviation			
	e	Source for the deviation is unknown			

NA: Not applicable

was considered.[8] One of the recommended quality practices to diagnose this error included calibration verification.[9] But till then, our laboratory had not adopted a practice of verification of calibration expect for IQC check after measurand calibration.

- Calibration verification was performed using IgG calibrators as testing materials obtained from Ortho Clinical Diagnostics.[9] These were a new set of similar calibrators that had been used for IgG calibration. Five calibrators of concentrations spanning the AMR were selected as testing materials. The samples were run in duplicates and the average of the observed values was compared against the assigned values (AVs) provided by the manufacturer by using:
- Difference plot

  A visual assessment of the data was done by using a difference plot which was created by comparing the observed difference (difference between the AV and the observed value) against the AV. The observed difference (%bias) seemed to be significantly high. This was followed by a statistical assessment of the difference plot which was done by comparing the observed bias against the allowable bias as per desirable biological variation (BV) specifications.[9,10] The observed %bias (8.79%) was greater than the allowable bias (4.3%) (Graph 1A).
- Linear regression plot

  A linear regression graph was plotted to find out the slope (Graph 1B), wherein slope describes the angle of the line that provides the best fit to the test and the comparative results, the ideal slope being 1.0. Slope is considered as an indicator for proportionate systematic error, wherein the magnitude of error increases as the concentrations get higher.

  Y-intercept describes the point where the line of best fit intersects with the Y-axis. Ideally, the Y-intercept should be 0. Y-intercept is considered as an indicator of constant systematic error that affects the comparability of results constantly across the measuring interval.

  The observed slope was 1.109. The observed slope was compared against the ideal slope (1.0). The criteria for acceptable performance were established as:

  Ideal slope ± TEa/100

  Acceptable performance = 1.0 ± (8/100) = 0.92 to 1.08.
- The observed slope showed a statistically significant positive deviation from the ideal slope suggesting a proportionate systematic error.[8] Hence, we performed a fresh calibration followed by calibration verification to ensure that this error was eliminated.

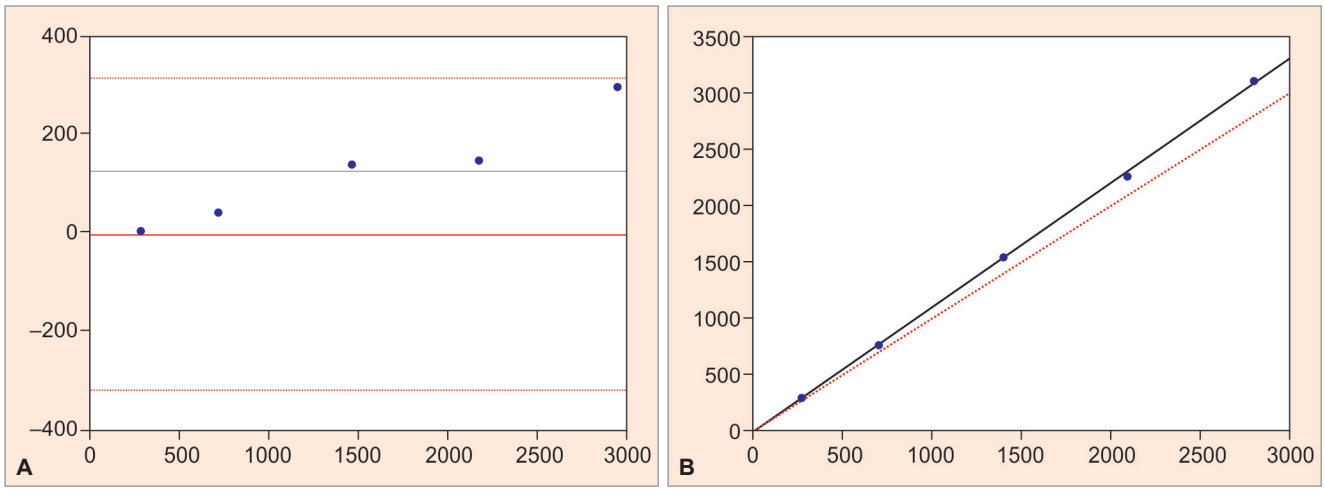
  This case showed how an EQA can guide us toward continual improvement of quality practice in the form of calibration verification.

Case 2

It was March 20, 2017, when EQA picked up a statistically significant "outlier" of serum CK-MB activity. External quality assurance (Bio-Rad Cardiac markers) reported CK-MB activity "outlier" in two equipments (Vitros 5600-1 and Vitros 5600-2) by CK-MB immunoinhibition method[11] with a Z-score of –3.85 and –3.92 in both the equipments.

Our laboratory did a structured root cause analysis for these outliers (Table 1). Based on the findings of the investigation, we zeroed in the possibility of a data handling error by the EQA provider.[7] The laboratory advocated possibilities of three types of errors with respect to handling of data by the EQA provider, which included:

- Inappropriate statistical procedure used by EQA provider for evaluation of dataset.



Graphs 1A and B: (A) Difference plot—IgG; (B) linear regression plot—IgG

- Error in establishment of "AV"
- Error in presentation of results by EQA provider.

Among these three errors, error (a) was ruled out, since the EQA whom we had enrolled with was an ISO 17043:2010 accredited provider and hence, the statistical procedures used by EQA provider were in compliance with international standard. We rule out error (c), which involved wrong presentation of results by EQA provider (when the EQA provider wrongly links the laboratory results for a specific method to another method). After having ruled out error (a) and (c) as possibilities, we zeroed in error (b) as a potential cause behind the outlier. This was based on the evidence that our laboratory results (from two equipments) were compared against the "AV" established by EQA in consensus with a group of participating laboratories not specific for our methodology. The gross discrepancy evident in CK-MB values when compared across methodologies by the EQA provider is explained by lack of metrological traceability of calibrators across different methods of CK-MB measurement.[11] This was the fourth instance in previous 6 months of EQA cycle, wherein CK-MB was branded outlier, based on comparison with AV not specific for our method though.

Assays are not standardized for measurands for which the calibrators are not traceable to a reference method or a reference material.[12] Hence, the laboratory has to evaluate the EQA results of such measurands against the method-specific consensus AV, if provided by the EQA and not the total group AV.

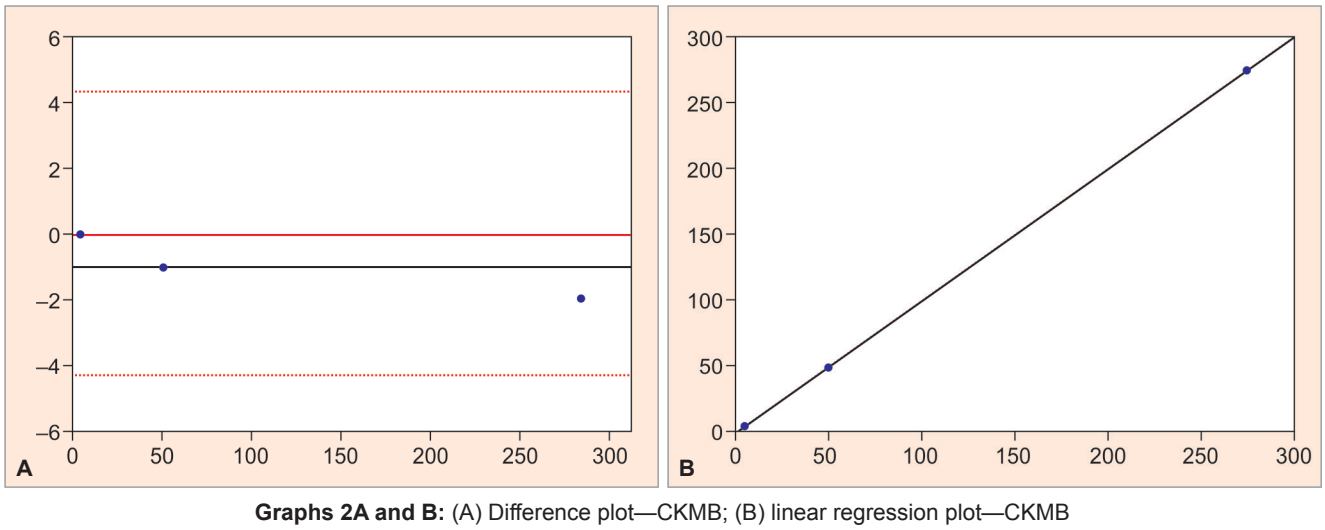
The EQA having declared CK-MB as an "outlier," but by comparing against a method nonspecific AV, we set out to gather evidence against the EQA to prove innocence of CK-MB activity including:

- In our laboratory, CK-MB EQA results were compared between two equipments (bias% is 0.29%). The comparison being made based on allowable %bias (7.1%) as per desirable BV specifications.[10]
- Calibration verification: Three CK-MB calibrators acquired from Ortho Clinical Diagnostics were used as testing materials.[9] The samples were run in duplicates and the average of the observed values was compared against the assigned values provided by the manufacturer by using a difference plot, wherein the observed bias (0.9%) was compared against the allowable bias (7.8%) as per desirable BV specifications.[9,10] The comparison yielded acceptable results (Graph 2A). This was followed by slope estimation by linear regression plot (Graph 2B). The observed slope (0.99) was compared against the ideal slope as per the criteria for acceptable performance (Ideal slope ± TEa/100, where TEa = 24.1%). The slope obtained from our study was within the acceptable limits (0.76 to 1.24) and hence, the analytical performance of CK-MB was considered acceptable.

This case illustrated a case scenario wherein EQA had falsely branded CK-MB activity as an "outlier" which was proved otherwise through detailed investigations by our laboratory.

Case 3

On May 31, 2017, the EQA provider (Bio-Rad) released the reports of analysis of clinical chemistry (monthly) program for measurands with serum-based matrix, which included serum copper (Vitros 5600, 3,5-Di-Br-PAESA 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-(3-sulphopropyl) aniline).[13] All measurands were reported to have acceptable performance according to the EQA provider including serum copper (Z-score: 1.63). Z-score < 2 is considered as an acceptable result according to ISO/IEC standard 17043:2010.[6] But our laboratory has adopted a quality practice of reviewing all EQA reports. The focus of our investigation turned toward serum copper EQA performance. Though, as per the EQA provider, copper showed an acceptable performance, we



Graphs 2A and B: (A) Difference plot—CKMB; (B) linear regression plot—CKMB

Table 2: Serum copper EQA result

Comparison	*n*	*Mean*	*Our result*	*SD*	*CV%*	*U*	*Z-score*	*Bias%*
Mode-based comparison*	52	63.9 µg/dL	95 µg/dL**	19.1 µg/dL	29.9%	6.63	1.63	48.7%

*Comparison against the total group AV, which is not specific to our methodology; **Vitros 5600, 3,5-Di-Br-PAESA 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-(3-sulphopropyl) aniline; CV: Coefficient of variation

were not satisfied with the comparison, since we observed a gross deviation of our value (95 µg/dL) from the AV of EQA (63.9 µg/dL) (Table 2).

The observed %bias between our value and AV of EQA (48.7%) was significantly greater than the allowable %bias (3.6%) as per desirable BV specifications.[10] But in contradiction, EQA showed an acceptable performance in terms of Z-score, against the total group AV, which was not specific to our methodology. Copper (in Vitros 5600) being a measurand without metrological traceability is bound to have an unreliable comparison across methodologies[12] unless compared against a higher order method like atomic absorption spectroscopy (AAS).[14] Though such a comparison was not available with the EQA provider, peer group analysis of labs performing copper with AAS was available for review by the EQA provider.

Hence, we tried to compare our EQA result against mean of EQA of labs performing AAS. The observed bias% was 2.8%, which was within the desirable bias% (3.6 %) according to BV desirable specifications. This comparison is done based on the laboratory's quality policy traceable to Clinical & Laboratory Standards Institute (CLSI) QMS 24-ED3:2016—"Using proficiency testing and alternative assessment to improve medical laboratory quality-3rd edition."[15] As per CLSI, when a clinical laboratory encounters a scenario, wherein it finds itself without a genuinely comparable peer group in EQA, and the EQA provider too is unable to grade its results, evaluation of these results must be done by the laboratory itself through comparison with an appropriate designate method in the EQA provider's summary report and in that case, the laboratory can use that method's mean and SD to do its own evaluation.

This investigation helped us to ascertain the quality of performance of serum copper in our lab. This case exposed the limitations of EQA which produced a contradictory report (with an acceptable Z-score, but an unacceptable %bias) and kept the laboratory guessing about the quality of its performance.

CONCLUSION

External quality assurance is an effective quality tool for accuracy assessment but with its own limitations, especially when it judges a laboratory's performance in comparison not against a reference method but with other participating laboratories. Hence, it is the need of the hour for the laboratory medicine specialists to understand the pros and cons of EQA and learn to interpret and troubleshoot EQA.

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