Spurious Outlier in Cell Counter for Total White Blood Cell Count Parameter in Laboratory External Quality Assurance Program

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Abstract

Background & Aims: In the External Quality Assessment Program (EQAP), medical laboratories' test results are compared to their peers by statistical analysis. The present study aims to highlight issues related to the root cause for the outliers in EQAP, the factors which lie outside the laboratory operation. **Material and Method:** Indian Society of Haematology and Blood Transfusion and All India Institute of Medical Science (ISHTM AIIMS) EQAP samples were analyzed in HORIBA Yumizen H 550 and HORIBA Pentra 60 cell counter. Randox international quality assessment scheme hematology (RIQAS) was analyzed on Yumizen H 550 cell counter. **Result:** All CBC parameters performance except Total WBC were found satisfactory as Z score is below three in ISHTM AIIMS EQAS on Yumizen H 550 in both laboratories. In contrast, Yumizen H 550 cell counter showed unsatisfactory performance as the Z score was more than three in both laboratories. In RIQAS Yumizen H 550 cell, the counter-performance of all CBC parameters is satisfactory for all samples as SDI is less than three. **Conclusion:** Horiba Yumizen H 550 cell counter gives spurious outlier in the total WBC parameter for ISHTM AIIMS EQAS. Switching to other EQAPs can provide the solution to a constant outlier.

Keywords: Cell counter, External quality assurance, Yumizen H 550, RQAS, Total WBC count

Introduction

Laboratories service users expect that laboratories shall provide reliable test results that would help them diagnose and manage their patients. The results should be reproducible and consistent from day to day and between laboratories to obtain comparable results[1].

There are a few terminologies of quality assurance system that needs to be broadly understood, such as Proficiency testing (PT). This term is more used in North America. PT's primary role is to evaluate laboratory performance for regulatory purposes. External Quality Assessment Schemes (EQAS) term routinely used in Europe and South America. EQAS focused on method and laboratory performance with an educational purpose. External Quality Assurance Program (EQAP) is an interlaboratory comparison that operates with one or more points like analytical performance, test interpretation, clinician advice for laboratory requests and diagnosis, method performance evaluation, and the vigilance of manufacturers, continuous education, training, and support. The primary intention of the activities of an EQAP in laboratory medicine is to support quality improvements of the services provided by participating laboratories for the benefit of the patients[2].

In EQAP or PT programs, the statistical analysis compares medical laboratories' test results with their peers' expected or target values. External Quality Assessment (EQA) participation and satisfactory performance are essential for medical laboratories seeking accreditation to the international standard ISO 15189:2012 Medical laboratories – Requirements for quality and competence[3]. EQA allows the comparison of results between laboratories and between method performances. Using EQA results, EQA users can identify methods with the most negligible bias, and worst bias as compared to the

target/ assigned mean used by that EQA provider to evaluate participant results. EQAP provider plays a vital role in maintaining and improving analytical quality and medical appropriateness of clinical laboratory data. For this, the EQAP organization shall provide samples like patient samples with relevant properties on which the diagnostic procedures will be performed[4.] The EQA samples should be handled as routine patient samples to get a fair comparison. EQA provider usually uses manipulated samples that make them resemble pathological samples. EQAS function differently in various countries. In some countries, EQA provides information based on which accreditation or licensing is allowed, and legal, financial, or professional sanctions are approved on laboratories by respective authorities. In some countries, only laboratories enrolled in an EQA scheme that produces satisfactory performance can charge fees or impanel in the health insurance agency. Good performance in an EQA scheme is key to recognizing them as a training center in a few countries[1.]

In India, accreditation or certification is voluntary and not compulsory for running a clinical laboratory. It is estimated that out of over 100000 clinical diagnostics laboratories, only 1038 medical laboratories are accredited according to ISO 15189 in India[5]. The complete blood count (CBC) is one of laboratory medicine's most frequently requested tests. Hematology automated cell counter utilizes impedance and light scattering techniques to know the size and physical characteristics of the blood cells, allowing their differentiation and counting[4.] CBC by automated cell counter is highly reliable and provide result in a quick time.

Randox international quality assessment scheme (RIQAS) is available for CBC parameters. In India EQAS for CBC by hematology cell counter is commonly provided by the Indian Society of Haematology and Blood Transfusion and All India Institute of Medical Science (ISHTM AIIM) and multinational commercial players like Randox and Bio-rad. ISHTM AIIMS EQAP started in the year 2003. More than 4500 participants to date enrolled. The program is based on the WHO international council for standardization in hematology: 2008, ISO/IEC 17043:2010 and ISO/IEC 13528:2015[6.] RIQAS hematology is since last 20-22 years. More than 7000 participants enrolled worldwide, and in India, 275 participants registered. RIQAS is accredited to ISO/IEC 17043:2010. The RIQAS assay material is liquid ready-to-use and 100% whole blood[7.] The cost-wise, ISHTM AIIMS EQAP is very cheap. It is about one-fifth of the cost of RIQAS hematology.

The performance of the participant lab in the EQA program is evaluated by comparing with the mean of peers or all participants for that parameter. Usually, the result is expressed as a Z or Standard Deviation Index (SDI) score. Suppose the Z score or SDI is more than three. Less than two scores mean performance is satisfactory for that parameter. If performance is unsatisfactory and needs evaluation and immediate action is required, Z score or SDI 2-3 performance is borderline attention required. Still, immediate action is not required.

The present study highlights the causes of a spurious outlier in EQA where the reason is not in laboratory operation. For the first time, a total two-year data analysis of two different EQAPs is available for cell counters of the same manufacturer with a different analytic approach.

Materials and Methods:

The present study is a retrospective analysis of two standalone medium-size laboratories in Western India, In laboratory 1 study is done on Horiba Yumizen H 550 (HORIBA Medical, Montpellier, France) and Horiba Pentra 60 (HORIBA Medical, Montpellier, France), and in laboratory 2 study is done on Yumizen H 550 cell counter. Both cell counters are maintained as per manufacturer protocol. Both laboratories are independent in lab operations and situated 20 miles from each other. For CBC parameters both laboratories enrolled in ISHTM AIIM EQAP and RIQAS hematology. ISHTM AIIM EQAP is giving four samples per year, one every quatre. RIQAS hematology gives twelve samples every year, one each month. The present study includes an analysis of samples of ISHTM AIIMS EQAP 2019-20 and samples of RIQAS hematology cycle 13 in 2020-21. For laboratory 1, March 2020, and for laboratory 2 March, April, May, December 2020, February, April, and May 2021 RIQAS hematology sample was not run due to a supply issue because of the COVID 19 pandemic situation so the analysis was not available. EQA material has been sent to us by the EQAP service provider through courier. The EQAP material is received only if it is found satisfactory on physical examination and temperature. The EQAP samples were analyzed as per supplier protocols in a patient mode without an autoloader. We have analyzed the report once it is available on web site or

physical copy provided by the EQAP provider. Microsoft office 365 excel was used for statistical analysis and graph preparation.

Results:

Table 1. Z score performance of Yumizen H 550 cell counter in ISHTM AIIMS EQAP

Parameter	Laboratory 1				Laboratory 2			
	Feb 2019	May 2019	Aug 2019	Nov 2019	June 2019	Sept 2019	Dec 2019	Sept 2020
RBCs	-0.09	0.99	0.10	1.10	0.50	0.31	-0.34	0.45
Hb	-0.96	-0.51	-1.35	0.51	2.86	-0.45	0.61	1.54
Hct	-0.67	0.61	-0.20	0.40	-0.55	1.08	0.14	0.49
MCV	-0.63	0.22	-0.13	-0.06	-0.53	0.41	0.37	0.15
MCH	-1.05	1.40	-1.08	-0.43	-1.84	-0.90	0.77	0.80
MCHC	-0.07	-0.65	-0.32	-0.15	-0.12	-1.30	0.15	1.63
Platelet count	2.07	-0.46	-0.45	1.87	0.19	-0.47	0.91	0.65

Abbreviations: RBCs, Red blood cells; Hb, Haemoglobin; Hct, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.

As shown in table 1, CBC parameters' namely RBCs, Hb, Hct, MCV, MCH, and MCHC performance were found satisfactory as the Z score is below three in both laboratories. Z score derived from target value derived from all participant lab results.

Table 2 Total WBC count (K/ul) performance on Yumizen H 550 and Pentra 60 cell counters in ISHTM AIIMS EQAP

Cell counter	Month and year	Lab Value	Consensus value (All lab)	Z score
Yumizen H 550 , Laboratory 1	Feb 2019	31.7	21.1	7.79
	May 2019	10.1	19.8	-5.21
	Aug 2019	05.4	09.3	-6.09
	Nov 2019	06.8	30.1	-16.4
Pentra 60 Laboratory 1	Feb 2019	21.4	21.1	0.22
	May 2019	21.5	19.8	0.91
	Aug 2019	09.1	09.3	-0.31
	Nov 2019	31.4	30.1	0.91
Yumizen H 550 Laboratory 2	Jun 2019	4.11	11.4	-7.37
	Sept 2019	2.62	09.6	-4.77
	Dec 2019	13.3	10.1	4.02
	Sept 2020	4.5	7.7	-5.45

As depicted in table 2 The total WBC count performance is satisfactory in Pentra 60 cell counter for ISHTM AIIMS EQAP as Z score is below three, while Yumizen H 550 showed unsatisfactory performance as Z score is more than three.

Table 3 Laboratory 1 performance by SDI score on Yumizen H 550 cell counter in RIQAS

Month and Year	RBCs	Hb	Hct	MCV	MCH	MCHC	Platelets count
Jan 2020	1.17	0.47	1.33	0.73	-0.62	-1.08	-1.11
Feb 2020	0.13	0.13	1.29	0.98	-0.34	-1.22	-0.92
April 2020	1.53	1.00	1.92	1.07	0.25	-1.16	-0.76
May 2020	0.66	0.57	0.70	0.34	0.09	-0.10	0.36
June 2020	0.92	0.91	1.34	0.59	0.18	-0.45	1.53
July 2020	0.72	0.38	0.23	-0.01	-0.14	-0.03	-0.07
Aug 2020	-0.03	0.46	0.67	0.55	-0.13	-0.28	-1.33
Sep 2020	1.07	0.76	1.13	0.55	-0.37	-0.65	2.23
Oct 2020	1.61	1.17	1.77	0.77	-0.14	-0.72	0.45
Nov 2020	1.71	0.65	1.86	0.89	-0.63	-1.04	1.07
Dec 2020	-0.08	0.12	1.80	2.09	0.36	-1.42	-0.32

Abbreviations: RBCs, Red blood cells; Hb, Haemoglobin; Hct, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.

As depicted in table 3 in Laboratory 1, Yumizen H 550 cell counter RIQAS performance of all CBC parameters was satisfactory as SDI is less than three.

Table 4 Laboratory 2 performance by SDI score on Yumizen H 550 cell counter in RIQAS

Month and Year	RBCs	Hb	Hct	MCV	MCH	MCHC	Platelets count
Jan 2020	0.3	-0.9	1.6	1.2	-0.51	-.077	-0.57
Feb 2020	0.0	-0.16	0.48	0.50	-0.53	-0.02	-0.32
June 2020	-2.35	-1.44	-0.76	0.53	0.63	-0.06	0.96
July 2020	-1.15	-0.25	-0.77	0.02	0.53	0.43	-0.50
Aug 2020	1.26	0.46	0.10	0.64	0.75	0.05	-0.59
Sep 2020	-0.33	-0.35	0.20	-0.18	-0.52	0.43	3.37
Oct 2020	-0.06	0.11	-0.10	-0.10	0.04	0.20	0.55
Nov 2020	-0.62	-0.16	0.16	0.59	0.10	-0.22	0.63
Jan 2021	0.16	-0.31	-0.24	-0.30	-0.17	0.09	1.04
March 2021	0.41	-0.70	0.44	0.12	-0.69	-0.89	0.79
July 2021	0.07	-0.03	0.16	0.11	-0.07	0.04	1.40

Abbreviations: RBCs, Red blood cells; Hb, Haemoglobin; Hct, Haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration.

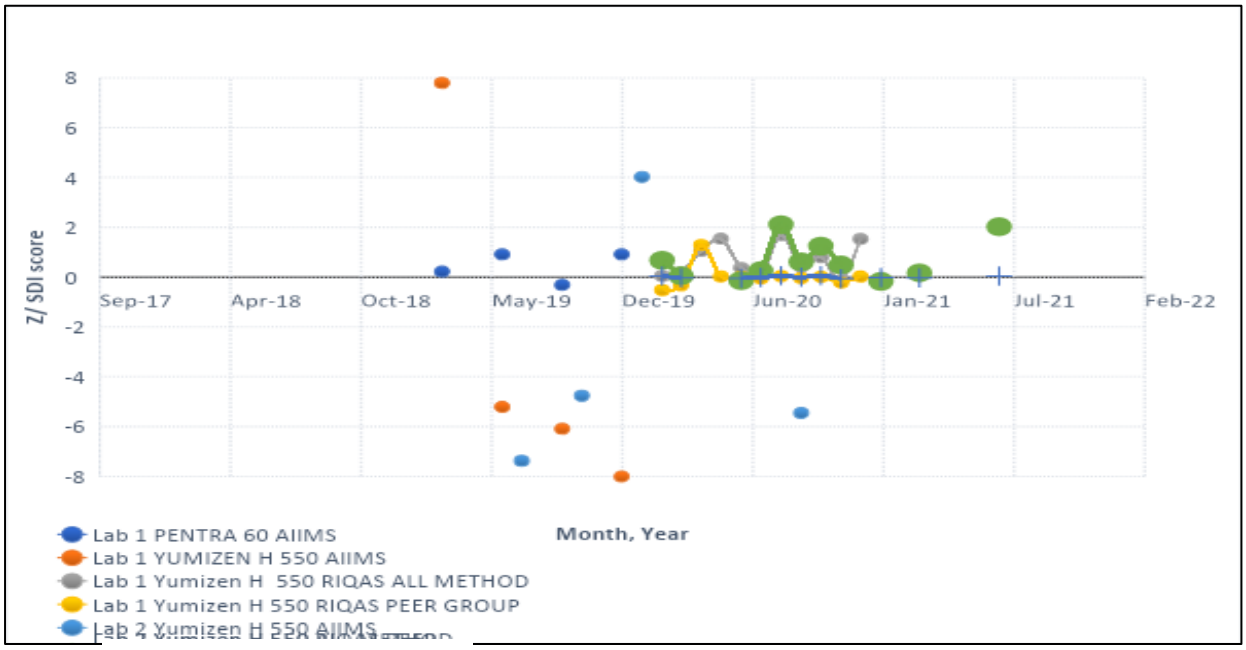
As depicted in table 4, in Laboratory 2, Yumizen H 550 cell counter RIQAS performance of all CBC parameters was satisfactory for all samples as SDI is less than three.

Table 5 Total WBC(K/ul) performance by SDI score on Yumizen H 550 in RIQAS hematology

	Laboratory 1			Laboratory 2			
Month and year	Yumizen H 550 value	Peer mean	SDI as peer	Month and year	Yumizen H 550 value	Peer mean	SDI as peer
Jan 2020	19.250	19.218	0.04	Jan 2020	19.80	19.218	0.67
Feb 2020	8.900	8.829	0.18	Feb 2020	8.82	8.82	0.05
April 2020	2.660	2.540	1.05	June 2020	8.70	8.75	-0.14
May 2020	2.550	2.385	1.54	July 2020	9.038	9.140	0.25
June 2020	8.910	8.755	0.37	Aug 2020	2.740	2.470	2.1
July 2020	9.080	9.038	0.10	Sep 2020	15.50	15.08	0.61
Aug 2020	2.660	2.472	1.69	Oct 2020	9.070	8.591	1.24
Sep 2020	15.370	15.083	0.42	Nov 2020	20.5	20.06	0.48
Oct 2020	8.900	8.591	0.80	Jan 2021	2.72	2.70	-0.18
Nov 2020	19.950	20.068	-0.13	March 2021	8.86	8.792	0.17
Dec 2020	2.590	2.424	1.53	July 2021	6.25	5.97	2.02

As per table 5, in laboratory 1 and laboratory 2, Yumizen H 550 cell counter total WBC showed no significant difference in SDI in peer-group means in RIQAS hematology. SDI for the peer group is satisfactory as SDI is less than three for all samples.

Figure 1 Total WBC performance in EQAS on Yumizen H 550 cell counter



As shown in figure 1, in ISHTM AIIMS EQAP performance is not satisfactory in Yumizen H 550 cell counter in both laboratories. In RQAS hematology total WBC performance is satisfactory in all cycles in both laboratories in the same cell counter.

Discussion:

EQA participation requires major laboratory staff and management efforts involving time, effort, and money. In the United States of America, all clinical laboratories doing complex tests must participate in EQA programs approved by the Centers for Medicare and Medicaid Services under the Clinical Laboratory Improvement Amendment Act (CLIA). If a laboratory gets 'Unsuccessful proficiency testing performance', it may suspend the CLIA certificate and Medicare payments. Suppose the laboratory fails to get a satisfactory performance for two consecutive or two out of three consecutive testing events. In that case, that laboratory is considered an unsuccessful performance in that test parameter[8].

When we got spurious outlier only in the total WBC count in Yumizen H 550 cell counter, we used a checklist for root cause analysis. The significant root cause points are sample integrity, equipment calibration, equipment repair and maintenance, internal control performance, and entry errors in the EQAP site. In each cycle, we checked each above points, but no conclusion was achieved in root cause analysis for ISH AIIMS EQAS Total WBC outlier in Yumizen H 550 cell counter. We went for cell counter-specific information. In 2019 Pentra 60 cell counter showed a satisfactory Z score for total WBC in ISHTM AIIMS. As the performance of all parameters except total WBC is satisfactory in both Yumizen H 550 cell counters in both laboratories.

Yumizen H 550 measures total WBC count by impedance, light scatters at 0 angles, light absorbance, and Double Hydrodynamic Sequential System technics (DHSS). Yumizen H 550 has two chambers, one for Hb, dilution, and the second for RBC and platelet. Yumizen H 550 uses three reagents, white diff, diluent, and cleaner, for analysis[9]. In Pentra 60 cell counter, total WBC is derived by impedance, light absorbance, cytochemistry principal, and DHSS. Pentra 60 uses four chambers dilution, Hb, RBC/platelet, WBC/baso, and Differential WBC for analysis. Pentra 60 uses five reagents, diluent, easophilfix, basolyse, lysebio, and cleaner, for analysis[10] . Meanwhile, we did not receive any negative feedback from users like treating doctors that results do not match a patient's clinical

condition. Interlab comparison with another local laboratory by patient sample for total WBC results in Yumizen H 550 was satisfactory. So, we thought that the cause of the outlier was not the cell counter. As we plan for accreditation, we changed CBC EQA from ISHTM AIIM to RIQAS hematology.

EQAP material can be prepared from EDTA anticoagulated blood, citrate dextrose phosphate anticoagulated blood, animal blood, or stabilized whole blood with an aldehyde[4] .Streck, Inc (*Omaha, Nebraska, USA*) [11] and R&D Systems, Inc (*Minneapolis, USA*) [12] are providing whole blood assay material for blood cell counting and are widely used in EQA. Stabilized blood, whether prepared or purchased commercially, is suitable for schemes with large numbers of participating laboratories and where prolonged delivery time is expected[4] . Our EQAP providers are ISHTM AIIMS, and RIQAS are well established and experienced. ISHTM AIIMS provide standard operating procedure on mail which mention they prepared EQA material by aldehyde stabilized whole blood. The method of EQA material preparation is not available to us via the website, kit insert, or instructions for users, representatives, etc. RIQAS said that it is proprietary.

Large participant EQAP providers usually use self-made stabilized whole blood or purchase it commercially. Large volumes of assay material can be prepared. Still, the fixation changes the cell membranes, making it non-commutable across different cell counter platforms of the same or different manufacturer, particularly for WBC, platelet count, and red blood cell means cell volume[13] .Yumizen H 550 cell counter showed satisfactory performance in all CBC parameters in RIQAS hematology and ISHTM AIIMS EQAP except total WBC in ISHTM AIIMS EQAP. This can be due to the assay material preparation method and related issues. Partially fixed and stabilized blood used for EQA causes improper separation of leucocytes and reticulocyte counts for some analyzers. EQAS providers should not select such assay materials, which are disadvantageous to an individual in vitro diagnostic medical device[14] . In the ISHTM AIIMS EQAP method, the preparation of EQA material is the primary cause for a spurious outlier performance of total WBC in the Yumizen H 550 cell counter. It is not commutable to Yumizen H 550, particularly for total WBC. Moreover, method preparation makes the material more vulnerable to temperature change during transport to the long-distance laboratory of 360 miles like ours by courier, which usually takes about 4-7 days for delivery.

ISHTM AIIMS EQAP does not provide peer consensus value. It gives all participant labs consensus value as a target, and RIQAS provides peer and all method consensus value. In the absence of a reference method target value, the results should be compared with a group of cell counters that use the same analytical technique. In consensus values, the most dominant group inaccuracy leads to bias[15]. Laboratories must use the EQA results to compare their results with peer consensus or reference method results, and it will lead to harmonizing their cell counter with a consensus group[16].

EQA samples are treated with chemicals to lengthen the shelf life, making them more vulnerable to transport-related problems like temperature fluctuations and vibration. Therefore, direct comparison with patient results is not possible. For RIQAS, peer method means, and all method means show no significant difference in all parameters of CBC. All Parameters of CBC other than total WBC performance are satisfactory in ISHTM AIIMS EQAP. So, the total WBC outlier in ISHTM AIIMS EQAP target value assignment is not the cause of the outlier.

The limitation of the present study is it involved only 2 laboratories' data but it will not impact the overall issue. Since Yumizen H 550 series cell counter is new and unique in the market, knowledge of the issue will help users to choose EQAP and answers the queries in the accreditation process.

Conclusion:

Horiba Yumizen H 550 cell counter gives spurious outlier in total WBC parameter for ISHTM AIIMS EQAS. EQA assay method preparation is essential for the root cause of the constant outlier. Switching to other EQAPs can provide a solution to constant outlier EQAP material that can be non-commutable to different cell counters. The EQAP provider shall disclose a list of such cell counters. The user manual of EQAS shall include a method of assay preparation EQAS providers evaluate their performance by taking participant feedback and incorporating report content requirements from international quality standards.

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