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ORIGINAL ARTICLE

Evaluation of Sysmex XT-2000*i*V analyzer performance across a network of five veterinary laboratories using a commercially available quality control material

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Abstract

Background: Laboratory and instrument harmonization is seldom reported in the veterinary literature despite its advantages to clinical interpretation, including the use of interchangeable results and common reference intervals within a system of laboratories.

Objectives: A three-step process was employed to evaluate and optimize performance and then assess the appropriateness of common reference intervals across a network of six Sysmex XT-2000*i*V hematology analyzers at 5 commercial veterinary laboratory sites. The aims were to discover if harmonization was feasible in veterinary hematology and which quality parameters would best identify performance deviations to ensure a harmonized status could be maintained.

Methods: The performance of 10 measurands of a commercially available quality control material (Level 2—Normal e-CHECK (XE)-Hematology Control) was evaluated during three 1-month time periods. Precision and bias were assessed with Six Sigma, American Society of Veterinary Clinical Pathology (ASVCP) total error quality goals and biologic variation (BV)-based quality goal approaches to performance measurement.

Results: Instrument adjustments were made to 1 analyzer twice and 3 analyzers once between evaluations to improve performance and achieve harmonization. Sigma metrics improved from 37/50 > 6 to 58/60 > 6 and to all >5 over the course of the harmonization project. BV-based quality goals for desirable bias and for laboratory systems of 0.33 × CV_I (within-subject biologic variation) were more sensitive and useful for assessing performance than the ASVCP total error goals.

Conclusions: Optimization and harmonization were achieved, and because BV-derived bias goals were achieved, common reference intervals could be implemented across the network of analyzers.

KEYWORDS
analytical performance, biologic variation, hematology, quality system, sigma metrics, Sysmex, veterinary

1 | INTRODUCTION

Interlaboratory or intralaboratory comparisons across multiple instruments in human clinical laboratories are well documented[1-4]; however, a few similar evaluations are found in the veterinary literature.[5] This could be attributed to a multitude of factors, including that human laboratory regulations are not applied to veterinary laboratories[5,6]; the results of comparative external quality assurance (EQA) performances are not routinely available and/or shared, and there is a lack of reporting of efforts or possibly an absence of efforts to harmonize results within or between veterinary laboratory businesses and networks. Harmonization of methods across laboratories means the standardization of results across methods and laboratories when no certified reference material is available. It is based on an agreed-upon standard for performance among participating laboratories to ensure that results have sufficient uniformity to be used interchangeably across a laboratory network for an individual patient.[3]

Synlab Veterinary Pathology Group (Synlab-VPG) operates six Sysmex XT-2000*i*V analyzers within their network of five laboratories located in the UK and Ireland, providing an environment in which to explore a veterinary hematology harmonization approach for the benefit of a group of veterinary clinical pathologists, client veterinarians, and ultimately, patients. Were a successful approach identified, it could provide a useful template to implement a harmonization process for the veterinary hematology community.

At the core of the harmonization process is a set of quality goals that are used to measure acceptable performance and progress toward harmonization. Such measures include error goals for veterinary hematology provided by the American Society for Veterinary Clinical Pathology (ASVCP)[7] or from internal expert opinion based on QC validation; canine biologic variation (BV) goals for bias and CV, and the canine BV goal for a laboratory system ($0.33 \times CV_I$).[8] Sigma metrics is also a useful indicator of performance where methods >6 sigma are considered to have "World Class" performance.[9]

The objectives of this study were to:

1. Evaluate the performance of the six Sysmex hematology analyzers within the five SYNLAB-VPG laboratories using a commercially available quality control material.
2. Optimize the performance of each analyzer to ensure the total error budget is contained within ASVCP guidelines or those determined by internal expert opinion.
3. Determine if BV goals for CV and bias and/or laboratory system performance would enable the use of common reference intervals.
4. Determine those quality goals that would be the most useful for identifying performance deviations that need to be addressed to maintain the interchangeability of results across the laboratory system.

We hypothesized that performance across laboratories would be similar since the same instruments were used, as well as the same reagents and quality control materials. We additionally hypothesized that the quality goals or a minimal deviation from the goals would be achievable.

2 | MATERIALS AND METHODS

2.1 | Analyzers

Six Sysmex XT-2000*i*V hematology analyzers (Sysmex Corporation) using veterinary software version 00-13 were compared among five veterinary sites within SYNLAB-VPG. These were designated as instruments one to five (site 4 has two instruments, designated as 4A and 4B). Ten measurands were selected for statistical quality performance analysis, as we considered them the most clinically relevant in current veterinary practice: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), platelet count (PLT), red cell distribution width-coefficient of variation (RDW-CV), plateletcrit (PCT), and reticulocyte number (RETIC). Additional measurands within the capability of the analyzer and represented in the quality control material (QCM) package insert were not included in the performance evaluation. The methods used to determine these measurands are summarized in Table 1 (SEED 2012[10] and Sysmex-2000*i*V/XT -1800*i*V user manual).

The Sysmex XT-2000*i*V hematology analyzer can be operated in open or closed mode; for this study, an open mode was used, ie, the probe aspirates the required volume from an open uncapped tube.

2.2 | Quality control material

A single level of QCM (level 2—Normal e-CHECK (XE)-Hematology Control) was analyzed once per day at instrument start-up, prior to the analysis of patient samples, by a fully trained technician according to a standard operating procedure. The QCM was refrigerated when not in use, and opened vials were used within 7 days. Performance was deemed acceptable for inclusion in the study data when the daily internal quality control (IQC) value was within the manufacturer's recommended acceptable range.

The manufacturer's target mean for the QCM was chosen for comparison across the group of analyzers.

2.3 | Data collection

Over three separate evaluation periods of November (Month 1), December 2019 (Month 2), and March 2020 (Month 5), each of the laboratories collected approximately 30 consecutive days of QCM result data from six Sysmex XT-2000*i*V analyzers. Only four sites (five analyzers) could participate in the Month 1 period due to an inability to obtain the Level 2 QCM. The data were exported to Microsoft Excel (Microsoft Excel for Mac 2011, Version 14.7.7 [170905] Last update 14.7.7). Each laboratory sent individual spreadsheets for analysis, and the data were used to calculate quality evaluation metrics. Intervention recommendations such as

TABLE 1 Methods[10] used by the Sysmex XT-2000*i*V hematology analyzer for determination of the hematologic measurands

Measurand (abbreviation) (units)	Method
Red blood cell count (RBC) ($\times 10^6/\mu L$)	Impedance direct-current detection with hydrodynamic focusing (number of electrical pulses generated per volume of blood)
Hemoglobin (HGB) (g/dL)	Cyanide-free, spectrophotometric method
Hematocrit (HCT) (%)	Impedance direct-current using cumulative pulse height
Mean cell volume (MCV) (fL)	Calculation: MCV (fL) = HCT/RBC
Mean cell hemoglobin concentration (MCHC) (g/dL)	Calculation: MCHC (g/dL) = HGB/HCT
Red cell distribution width–coefficient of variation (RDW–CV) (%)	Histogram generation from impedance
Reticulocyte Count (RETIC NUMBER) ($\times 10^6/\mu L$)	Fluorescence flow cytometry
White blood cell count (WBC) ($\times 10^3/\mu L$)	WBC-BASO channel method for total WBC count using forward and side scatter light (SCFSC)
Platelet count (PLT) ($\times 10^3/\mu L$)	Impedance with hydrodynamic focusing
Plateletcrit (PCT) (%)	Impedance with hydrodynamic focusing

optimal QCM handling and instrument adjustments and/or service were applied between each evaluation period based on the results obtained and the likely root causes for the detected deviations in performance.

2.4 | Quality evaluations

To evaluate the observed analytical imprecision and bias, the SD and CV[11] bias in units and absolute bias %[12] from the manufacturer's QCM target mean were calculated as described previously[13] for all 10 measurands from each analyzer for each evaluation period.

From the initial calculations for imprecision (CV%) and bias (absolute bias %), the total error observed (TE_{obs}) was calculated and expressed as a percentage to allow comparability with analytical quality requirements[14] using the following formula[7,14];

$$TE_{obs}\% = |\text{Bias}\%| + 2 \times CV\%$$

A sigma metric approach was taken to estimate the clinical performance of the 10 measurands across all sites using the following formula,[9,15] and Total Error Allowed (TEa) from Table 2.

$$\text{Sigma metric} = (TEa\% - |\text{Bias}\%|)/CV\%$$

The sigma metric was chosen as an important reflection of analytical capability, allowing a comparison across methods. It is considered to be a reflection of risk associated with an analytical process and correlates with the "Westgard Sigma Rules."[16,17] According to Westgard, measurands with 6 sigma or greater performance are easily controlled with a simple quality control (QC) rule and low numbers of QCM data, while those with 5-6 sigma can be controlled with a short multirule ($1_{3s}/2_{2s}/R_{4s}$) and low numbers of QCM data. Those methods with 4-5 sigma performance can be controlled with the addition of a fourth rule to a multirule ($1_{3s}/2_{2s}/R_{4s}/4_{1s}$). Those methods with 3-4 sigma performance require an even more complex multirule with larger numbers of QCM data but still may not achieve desirable levels of error detection.[12] The sigma metric, by definition, will change if performance changes, reflecting fluctuations in the observed CV and bias over defined time intervals.

For measurands with a sigma metric <6, a quality goal index (QGI) was calculated using the following formula:

$$QGI = |\text{BIAS}\%| / (1.5 * CV\%).$$

This calculation allowed us to determine whether imprecision and/or inaccuracy affected the overall quality goals using the following interpretative guidance: a QGI < 0.8 was considered indicative of imprecision while a QGI > 1.2 was indicative of inaccuracy (bias). QGI results between these two boundaries indicated both bias and imprecision contributing to <6 sigma performance.[18]

BV goals were applied as three additional performance indicators: BV-based CV, BV-based bias, and a BV-based laboratory system goal.[8] Canine BV goals were chosen because canine specimens are the most common submissions across all of the five laboratories. We calculated optimal, desirable, and minimum values for TE, CV, and bias goals based on canine BV data[14] for applicable measurands and $0.33 \times CV_I$ as the goal for multiple instruments within a laboratory system.[8] The $0.33 \times CV_I$ goal allowed us to detect differences between the means of serial measurements for each measurand to ensure comparability by checking to make sure the allowable difference was $<0.33 \times CV_I$.[8] The $\pm 0.33 \times CV_I$ goal was compared with the manufacturer's target mean for the QCM.

PCT and RET did not have BV data available.

We used the following formulas[8]

BV CV goals:

$$\text{Optimal CV: } < 0.25\,(CV_I)$$
$$\text{Desirable CV: } < 0.5\,(CV_I)$$
$$\text{Minimally acceptable CV: } < 0.75\,(CV_I)$$

BV bias goals:

TABLE 2 Quality goals for the evaluation of the performance of Sysmex XT-2000*i*V hematology analyzers for selected measurands

Measurand	ASVCP total error quality goals[a] or internal expert opinion goal (Bold) (%)	Canine biologic variation goals for coefficient of variation[b] (%) < Opt/Des/MA	Canine biologic variation goals for absolute bias[c] (%) < Opt/Des/MA	Canine biologic variation laboratory system goal ($<0.33 \times CV_I$) (%)
Red blood cell count	10	1.38/2.75/4.13	0.88/1.76/2.64	1.815
Hemoglobin	10	1.48/, 2.95/4.43	0.94/1.89/2.83	1.947
Hematocrit	10	1.60/3.20/4.80	1.03/2.06/3.09	2.112
Mean cell volume	7	0.525/1.05/1.58	0.418/0.836/1.25	0.693
Mean cell hemoglobin concentration	10	0.650./1.30/1.95	0.353/0.706/1.059	0.858
Red cell distribution width–coefficient of variation	10	1.00/2.00/3.00	0.901/1.80/2.	1.32
Reticulocyte number	**40**	N/A	N/A	N/A
White blood cell count	15	3.03/6.05/9.08	2.16/4.31/6.47	3.99
Platelet count	20	3.50/7.00/10.50	2.58/5.17/7.49	4.62
Plateletcrit	**25**	N/A	N/A	N/A

Note: N/A, no information for canine biologic variation available.

Sigma metric = (TEa% − Abs Bias%)/CV%.

[a]Nabity et al (2017).

[b]Biologic variation goals. CV: Opt = optimal, Des = desirable, MA = minimally acceptable. Optimal CV: $<0.25(CV_I)$; desirable CV: $<0.5(CV_I)$; minimally acceptable CV: $<0.75(CV_I)$.

[c]Biologic variation bias goals: optimal bias: $<0.125 \times (CV_I^2 + CV_G^2)^{1/2}$; desirable bias: $<0.25 \times (CV_I^2 + CV_G^2)^{1/2}$; minimally acceptable bias: $<0.375 \times (CV_I^2 + CV_G^2)^{1/2}$.

$$\text{Optimal bias: } <0.125\left(CV_I^2+CV_G^2\right)^{1/2}$$

$$\text{Desirable bias: } <0.25\left(CV_I^2+CV_G^2\right)^{1/2}$$

$$\text{Minimally acceptable bias: } <0.375\left(CV_I^2+CV_G^2\right)^{1/2}$$

Within-subject BV is denoted as CV_I, while between subject variation is CV_G.

3 | RESULTS

3.1 | Sigma metrics and QGI

3.1.1 | Initial evaluation of five Sysmex XT-2000*i*V analyzers Month 1

All of the hematology measurands chosen for this study for Analyzer 1 and Analyzer 2 had sigma metrics >6. Of the remaining three analyzers, Analyzer 5 had only 7, and Analyzers 4A & 4B had only five measurands >6 sigma (Table 3a).

3.1.2 | QGI Month 1

The QGI interpretation for those measurands performing <6 sigma is summarized in Table 4. Bias was more often a concern (14/24) than imprecision (8/24). Both bias and imprecision accounted for two occurrences.

Adjustments with instrument service at Analyzers 4A, 4B, and 5 were conducted to help eliminate bias. Improved control material handling was instituted across all laboratories before additional evaluations were undertaken in Month 2.

3.1.3 | Follow-up Month 2 and Month 5 evaluation on six Sysmex XT-2000*i*V analyzers

As demonstrated in Table 3b,c, sigma metrics generally improved over the following two evaluation periods, resulting in all but two measurands at one site (Analyzer 4A) achieving >6 sigma. All sites and all measurands achieved sigma >5. An instrument service visit was required for Analyzer 3, and an additional visit was required for Analyzer 5 to achieve this improvement in performance. QGI review of the remaining two measurands on Analyzer 4A identified imprecision for PLT, and bias was implicated for RBC (Table 4).

3.2 | Biologic variation goals

3.2.1 | Initial evaluation of BV goals on five Sysmex XT-2000*i*V analyzers

Achievement against CV, bias, and 0.33 × CVi goals could only be assessed for those eight measurands for which canine BV data were available (Table 2). No BV data were available for PCT and RETIC.

Analytical CVs achieved optimal or desirable CV goals for Analyzers 1 and 2. Optimal or desirable CV goals were achieved for seven of the eight measurands for Analyzer 5, and six of the eight measurands for Analyzer 4A and Analyzer 4B (Table 5a).

Optimal or desirable bias goals based on BV were achieved for seven measurands for Analyzer 1. Analyzer 2 was able to achieve optimal or desirable bias goals based on BV for six measurands. Analyzers 4A and 4B were able to achieve optimal or desirable bias goals for two and four measurands, respectively. Analyzer 5 was able to achieve optimal or desirable bias goals for three measurands but failed to achieve minimally acceptable bias goals for all other measurands (Table 5b).

For Analyzer 1, the $0.33 \times CV_I$ goal for laboratory system performance for QCM was achieved for seven measurands for which BV information is available. For Analyzer 2, the $0.33 \times CV_I$ goal for lab system was achieved for all measurands except PLT. For Analyzer 4A, the goal was achieved for three of the eight measurands (WBC, MCV, MCHC) but not for the remaining measurands. For Analyzer 4B, the $0.33 \times CV_I$ goal was achieved for five measurands but not for HGB, HCT, and RDW-CV. For Analyzer 5, the $0.33 \times CV_I$ goal was achieved for two out of eight measurands (MCV, MCHC) (Table 5c).

3.2.2 | Follow-up evaluation of BV goals on six Sysmex XT-2000*i*V analyzers in months 2 and 5

Analyzer 3, included in Month 2, achieved optimal or desirable CV goals based on BV for six of the eight measurands and all eight measurands in Month 5. The others sites achieved optimal or desirable CV goals for the eight measurands at both follow-up evaluations (Table 5a).

Analyzer 1 achieved optimal or desirable bias goals for the same seven measurands for Month 2 and Month 5. Analyzer 2 achieved optimal or desirable bias goals for eight measurands for Month 2, and 6 measurands for Month 5. Analyzer 3 achieved optimal or desirable bias goals for five measurands for Month 2, and all eight measurands for Month 5.

Analyzers 4A and 4B improved during Month 2, and Analyzer 4A met optimal or desirable bias goals for seven measurands where biologic data were available for Months 2 and 5. Analyzer 4B achieved optimal or desirable bias goal for all eight measurands in Month 2, but only achieved six of eight measurands in Month 5.

Analyzer 5, in contrast to Month 1, achieved optimal or desirable bias goal for seven measurands for Month 2, and six measurands for Month 5 (Table 5b).

The $0.33 \times CV_I$ goal was achieved by Analyzer 1 for six measurands; it did not achieve this goal for two measurands in Month 2, but this resolved by Month 5 when the goal was achieved for eight measurands. Analyzer 2 could not achieve the goal for 2 measurands in Month 2, but in Month 5 achieved this goal for six of eight measurands (but not for HCT and MCHC).

Analyzer 3 achieved the goal for 5 of 8 measurands in Month 2. This increased to 6 of 8 measurands in Month 5.

TABLE 3 Summary of sigma metrics for selected measurands for six Sysmex XT-2000*i*V hematology analyzers within a system of veterinary laboratories over three periods of evaluation

	Analyzer					
Measurand	**1**	**2**	**3**	**4a**	**4b**	**5**
(a) Initial Month 1						
WBC	6.54	10.64	N/A	9.76	9.38	8.35
RBC	15.21	13.00	N/A	3.88	5.51	7.10
HGB	7.24	23.00	N/A	6.00	2.82	5.57
HCT	9.38	12.70	N/A	3.48	4.92	4.08
MCV	11.50	12.20	N/A	12.34	12.40	8.15
MCHC	9.28	10.90	N/A	5.65	9.37	8.12
PLT	6.15	9.05	N/A	7.35	9.7	16.97
RDW-CV	18.00	25.36	N/A	0.47	0.596	2.03
PCT	6.06	8.56	N/A	6.50	7.75	7.00
Retic. No.	6.20	7.63	N/A	3.20	4.57	7.03
N σ > 6	10	10	N/A	5	5	7
N σ 5.0-5.9	0	0	N/A	1	1	1
N σ 4.0-4.9	0	0	N/A	0	2	1
N σ 3.0-3.9	0	0	N/A	3	0	0
σ N < 3	0	0	N/A	1	2	1
Intervention	None	None	N/A	ISA	ISA	ISA
	Improved QCM handling					
(b) Follow-up Month 2						
WBC	14.12	6.81	8.87	6.80	7.13	9.53
RBC	15.11	14.77	11	13.96	12.10	17.75
HGB	19.94	12.68	12.82	14.83	13.54	20.74
HCT	2.92	13.14	2.20	9.19	12.77	10.94
MCV	16.68	13.44	5.20	12.74	12.83	8.02
MCHC	14.13	12.05	5.71	10.15	9.53	11.32
PLT	8.07	7.62	4.94	9.73	6.29	6.94
RDW-CV	20.40	20.40	13.64	18.68	17.71	24.71
PCT	7.61	9.92	4.55	9.01	6.43	7.06
Retic. No.	5.59	6.48	7.83	5.59	7.10	5.04
N σ > 6	8	10	5	9	10	9
N σ 5.0-5.9	1	0	2	1	0	1
N σ 4.0-4.9	0	0	2	0	0	0
N σ 3.0-3.9	0	0	0	0	0	0
σ N < 3	1	0	1	0	0	0
Intervention	None	None	ISA	None	None	ISA
(c) Follow-up Month 5						
WBC	7.62	7.78	6.41	9.22	7.97	10.34
RBC	14.22	18.09	14.2	5.24	12.01	9.34
HGB	15.97	24.58	30.04	17.50	18.45	10.08
HCT	13.82	13.92	9.05	15.05	16.10	7.85
MCV	13.60	14.91	10.07	11.12	10.67	8.43
MCHC	13.50	13.12	8.51	10.57	12.55	10.70
PLT	7.60	9.05	6.80	5.12	7.30	7.65

TABLE 3 (Continued)

	Analyzer					
Measurand	1	2	3	4a	4b	5
RDW-CV	19.17	13.10	19.53	14.40	16.52	14.55
PCT	7.42	7.57	7.35	7.9	7.9	8.04
Retic. no.	6.90	8.00	8.50	7.60	6.00	8.35
N σ > 6	10	10	10	8	10	10
N σ 5.0-5.9	0	0	0	2	0	0
N σ 4.0-4.9	0	0	0	0	0	0
N σ 3.0-3.9	0	0	0	0	0	0
σ N < 3	0	0	0	0	0	0

Abbreviations: 1, November; 2, December; 3, March; HCT, hematocrit; HGB, hemoglobin; ISA, Instrument service and Adjustment; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; N, number; N/A, not available due to inability to source QCM; PCT, plateletcrit; PLT, platelet; QCM, quality control material; RBC, red blood cell; RDW–CV, red cell distribution wide–coefficient of variation; WBC, white blood cell.

Analyzer 4A achieved the goal for 7 of 8 measurands in Month 2 but by Month 5 achieved goals for all 8 measurands. Analyzer 4B achieved the goal for 6 measurands in Month 2 and achieved the goal for 7 of 8 measurands in Month 5, except for HCT.

Analyzer 5 achieved the goal for 6 of 8 measurands in Month 2. In Month 5, 7 measurands achieved this goal, apart from MCHC (Table 5c).

3.3 | Comparison of performance measures

Tables 5a, 5b, and 5c summarize the BV goals for bias, CV, and 0.33 × CV_I goal for laboratory system performance for QCM that reached optimal or desirable acceptable goals for 8 measurands where BV data are available, assessed during three evaluations. BV goals for bias and the 0.33 × CV_I goal for laboratory systems proved discriminatory when compared with the ASVCP goals, or expert opinion where sigma metrics was <6 sigma.

4 | DISCUSSION

This is the first report comparing six Sysmex analyzers in a network of five veterinary laboratories in veterinary peer-reviewed literature. We hypothesized that performance across the laboratories would be similar and would have minimal deviations from quality goals. This was true for some sites, but this study also demonstrated that 4 of the 6 analyzers initially exhibited differences in performance, despite initial harmonization efforts, ie, using the same instruments, reagents, QCM, processing frequency of QCM, and the same technical training. This study demonstrated that at certain times, Sysmex hematology analyzers might fail to meet quality requirements,[13] particularly for the erythroid measurands.

To monitor any degree of individuality for a network of Sysmex analyzers, we found sigma metrics to be a valuable indicator of performance-related issues. We decided to use this approach for a number of reasons. It is a universal benchmark that lends itself well to multiple site evaluation and continuous comparison. It incorporates both bias and imprecision, and as sigma metric values decrease, failure of the QC rule increases.[19] We also used quality goals based on BV to minimize analytical noise in serial results and ensure that bias and/or imprecision would not mask physiological changes.[8,20] This might also be useful for ongoing patient monitoring and the continued use of common RI within this network of laboratories.

We calculated 170 sigma metrics from 10 measurands (Table 3a-c), and from those, 24 (14%) did not achieve >6 sigma for "world-class" performance. For these, the QGI was useful for analyzing potential problems with those measurands by predicting whether the underperformance was due to imprecision, bias, or both, and informing the choice of intervention and adjustment remedies.

Bias was more likely to be a contributing factor than imprecision based on the QGI (58% of <6 sigma occurrences). The high degree of bias in comparison with imprecision was not unexpected, as managing bias is a regular occurrence in a clinical laboratory, but we needed to ascertain how the range of bias affected the sigma metric value. Where bias was problematic, more than half of the occurrences did not meet the minimally acceptable BV bias goals and/or the 0.33 × CV_I goal for QCM. However, the inability to achieve the minimally acceptable BV bias goal was not unique to measurands with <6 sigma. We found examples where BV bias goals were not achieved but sigma metrics were >6. This was because the bias was much lower than the total allowable error goal (based on ASVCP or expert opinion). When bias was a larger proportion of the TEa, the sigma value was <6; for these measurands, the bias ranged from 3.22% to 8.27%. Our evaluation suggests that bias >3% on the Sysmex XT-2000*i*V analyzer indicates an increased likelihood that the sigma metric value will be below "world-class" performance and have an increased likelihood to fail at least one or, in most cases, two of the quality goals (Desirable BV bias and 0.33 × CV_I goals) of the three biologic variation-based goals. For these occurrences, the imprecision varied from 0.89 to 3.61 (CVobs). Additional performance evaluations are needed to confirm the suggestion from these results that observed bias results >3.0% could be used to determine

TABLE 4 Summary of the investigation of measurands with sigma metrics <6 for 6 Sysmex XT-2000*i*V hematology analyzers in a system of veterinary laboratories using commercially available quality control material

Site	Measurand	Sigma metric	QGI	Interpretation of QGI	CVobs %	Achieved CV_A BV goal	Bias-obs %	Achieved bias BV goal	Achieved $0.33 \times CV_I$ goal
1	RETIC	5.59	0.69	Imprecision	6.04	N/A	6.253	N/A	N/A
	HCT	2.92	1.53	Bias	3.18	YES - Des	0.72	YES - Opt	YES
3	HCT	2.20	6.56	Bias	3.08	YES - Des	3.20	NO	YES
	MCV	5.20	0.16	Imprecision	1.31	YES - MA	0.19	YES - Opt	YES
	MCHC	5.71	2.21	Bias	1.30	Yes- MA	2.542	NO	NO
	PLT	4.94	2.597	Bias	3.84	YES - Des	1.01	YES - Opt	YES
	PCT	4.55	0.04	Imprecision	5.42	N/A	0.32	N/A	N/A
4a	PLT	5.12	0.17	Imprecision	3.72	YES - Des	0.93	YES - Opt	YES
	RBC (Month 1)	3.88	1.22	Bias	1.75	YES - Des	3.22	NO	NO
	HCT	3.48	1.32	Bias	1.70	YES - Des	4.06	NO	NO
	RDW-CV	0.47	1.52	Bias	3.61	NO	8.27	NO	NO
	RET	5.59	0.77	Imprecision	5.92	N/A	6.87	N/A	N/A
	RBC (Month 5)	5.24	6.67	Bias	1.60	YES - Des	1.60	YES - Des	NO
	MCHC	5.65	0.56	Imprecision	1.43	YES - MA	1.87	NO	YES
	RET	3.20	1.13	Both imprecision and bias	8.15	N/A	13.90	N/A	N/A
4b	RBC	5.51	0.55	Imprecision	1.57	YES - Des	1.32	YES - Des	YES
	HGB	2.82	1.66	Bias	1.88	YES - Des	4.69	NO	NO
	HCT	4.92	0.99	Both Imprecision and bias	1.56	NO	2.33	YES - MA	NO
	RDW-CV	0.60	1.75	Bias	3.10	NO	8.15	NO	NO
	RET	4.75	53.71	Bias	5.61	N/A	14.37	N/A	N/A
5	HGB	5.57	3.77	Bias	0.89	YES - Opt	5.04	NO	NO
	HCT	4.08	1.72	Bias	1.49	YES - Opt	3.88	NO	NO
	RETIC	5.04	0.29	Imprecision	7.29	N/A	3.217	N/A	N/A
	RDW-CV	2.03	27.75	Bias	1.61	YES- Des	6.71	NO	NO

Note: Tools used for the investigation included Quality Goal Index and Relationships of Observed Coefficient of Variation, Observed Bias, and Canine Biologic Variation-based Quality Goals.

QGI, Quality Goal Index (%) = Abs Bias (%)/1.5 CV (%), Interpretation: The QGI reflects the extent to which both bias and precision meet their respective quality goals without considering goals for CV and bias as separate entities. The QGI is interpreted as follows: QGI <0.8 = imprecision and QGI >1.2 = inaccuracy, whereas QGI of 0.8-1.2 = both imprecision and inaccuracy.

Abbreviations: Bias-obs %, observed analytical absolute bias (%); BV, biologic variation; CV, within-subject biologic variation coefficient of variation; CV_A, analytical coefficient of variation; CVobs %, observed analytical coefficient of variation; Des, desirable; HCT, hematocrit; HGB, hemoglobin; MA, minimally acceptable; MCV, mean cell volume; N/A, not available for this measurand; Opt, optimal; RBC, red blood cell count; RDW-CV, red cell distribution width-coefficient of variation; RET, reticulocyte number.

emerging or established problems and whether instrument servicing is indicated when these occur.

The $0.33 \times CV_I$ goal was not achieved for 11 out of 18 occurrences with the sigma metric <6; nine of these were during the initial phase, with one occurrence during the second phase and one during the third phase. Ten occurrences were attributed to bias, and one was due to both imprecision and bias. The ongoing attention to QC, QCM handling, and emphasis on QCM performance, as well as instrument servicing when excessive bias and/or low sigma metrics (<5) were identified, likely contributed to the increasing ability in achieving the BV-based laboratory systems goal over the three periods of evaluation. Continued monitoring of performance is needed to determine if this improved level of performance will be sustained. The $0.33 \times CV_I$ goal proved to be a good discriminatory goal for poor performance based on comparison with the QCM target.

From the initial evaluation, it was clear that analyzers 4A & 4B and Analyzer 5 required attention from Sysmex technical support, as nearly half of the measurands had sigma metrics <6 sigma during this phase and for the first assessment for Analyzer 3 in Month 2 where half of their measurands were <6 sigma. This was seen particularly in the erythroid measurands and following a Sysmex service call. We

TABLE 5A Summary of CV biologic variation goals that reached optimally or desirably acceptable levels for eight measurands where biologic variation data were available and assessed during three evaluations

	CV BV goal		
Analyzer number	Month 1	Month 2	Month 5
1	8	8	8
2	8	8	8
3	N/A	6	8
4A	6	8	8
4B	6	8	8
5	7	8	8

Note: Not applicable (N/A) is for an analyzer that was not using correct quality control material during the first evaluation.

Abbreviation: CV BV, coefficient of variation biologic variation.

TABLE 5B Summary of bias biologic variation goals that reached optimally or desirably acceptable for eight measurands where biologic variation data are available and assessed during three evaluations

	Bias BV goal		
Analyzer number	Month 1	Month 2	Month 5
1	7	7	7
2	6	8	6
3	N/A	5	8
4A	2	7	7
4B	4	8	6
5	3	7	6

Note: N/A is for an analyzer that was not using correct quality control material during the first evaluation.

Abbreviation: BV, biologic variation.

TABLE 5C Summary of $0.33 \times CV_I$ biologic variation goal for multiple instruments within a laboratory system that reached optimal or desirable, acceptable goals for eight measurands where biologic variation data are available and assessed during three evaluations

	$0.33 \times CV_I$ BV goal		
Analyzer number	Month 1	Month 2	Month 5
1	7	6	8
2	7	6	6
3	N/A	5	6
4A	3	7	8
4B	5	6	7
5	2	6	7

Note: Not applicable (N/A) is for an analyzer that was not using the correct quality control material during the first evaluation.

Abbreviation: BV, biologic variation.

saw improvements in those measurands in terms of sigma metrics which were now >5 sigma, and many were >6 sigma on subsequent evaluations. The performance variations and need for bias corrections were discussed with the service representative prior to making adjustments.

We standardized QCM handling among the sites, ensured routine maintenance procedures were adhered to and documented in the analyzer maintenance log, and improved the technicians' understanding of QC evaluation for identifying shifts, trends, and out of control results. Following these approaches, we observed improved sigma metrics across the three phases of QC data collection.

From this study, sigma metrics <5, whether attributed to bias or imprecision, did not meet all quality goals that were set. Sigma metrics >6 had better capabilities for reaching the BV goals and continuing improvements as these measurands had fewer QCM failures. Sigma metrics <4 were also unacceptable as these measurands could only achieve 1 out of the 3 BV quality goals and from our study required technical attention.

This raises the question as to whether there is a hierarchy of quality goals that should be used to achieve harmonization. We found the choice of BV goals used in this study to be sufficient for challenging the performance of the measurands. We also found that the TE or expert opinion goals were not discriminatory since all analyzers were able to achieve these goals, even when observed performance and sigma varied widely. Collected bias and imprecision data were more usefully applied to check the achievement of the BV goals rather than TE or expert opinion goals that were not major contributors to the improvement process. BV goals were superior, in that once desirable BV-based CV goals were met we could be confident that the patient results would not be misclassified clinically, and imprecision would not mask physiological change, which is reassuring for continued patient monitoring. We could also be assured that we could continue to use common reference intervals as long as we could meet the minimally acceptable BV bias goal.[8,21]

A limitation to using BV is that it varies with measurand. Thus, the permissiveness or stringency of the BV quality goals varies according to measurand[14]; however, a single numerical quality goal is not suitable across all measurands, which have different interpretations and clinical utilities, as well as differences in analytical performance. Despite this limitation, our study demonstrated that we could achieve optimal or desirable BV quality goals for most measurands and meet minimally acceptable goals for some measurands with the accumulated data from the three evaluations. From our final evaluation (Month 5) after the recommended improvements from the first two datasets, we met these BV goals most of the time. Where we did not meet the BV goals (25 occurrences across all evaluations <6 sigma), the most serious concern was the risk of masking within-patient changes due to analytical variation. Ongoing evaluations are needed to determine if sigma metrics alone or in combination with absolute observed bias and BV-based goals (especially $0.33 \times CV_I$ goal) can be reliably used on an ongoing basis for monitoring performance.

This study demonstrates that these quality specifications are achievable and that harmonization is possible. However, fluctuations in the ability to achieve these goals and in the sigma metrics were seen throughout the three evaluations for some sites, while others remained relatively consistent. These differences could be due to inherent analytical variability between the analyzers, even though all were of the same model by the same manufacturer. They could also be due to the capability and training of the operators and environmental conditions at each site, and the degree of quality management engagement and varying analyzer throughput could also have contributed. A publication from Sysmex[10] also made reference to this by quoting analyzer differences from the same manufacturer will still have some disparity and also indirectly highlighted the need to further scrutinize calculated measurands. MCHC is derived from HGB and HCT values,[10] and so its validation must be compared with their performances; for example, when MCHC was <6 (Table 3b, Analyzer 3), the corresponding Sigma metrics for HCT and HGB were 2.20 and 12.82, respectively, indicating that the poor MCHC performance could likely be attributed to poor HCT performance.

From our experience to date, in conjunction with BV bias and the 0.33 × CV_I goal, we recommend using a sigma-based quality management system as the method to monitor and compare performance in a network of laboratories; however, this should be carried out on a continuous and routine basis to detect any changes in performance. The application of sigma metrics to assess and compare instruments, seen for chemistry and immunoassays methods in human laboratories, demonstrated that a sigma metric <4 would cause QC rules to fail 20 times more frequently than methods with a 6 sigma metric.[19] Additionally, for the better-performing methods, the cost of supplies and reagents was significantly lower.[4,19] More specifically, a study of sigma metrics in human laboratories using the Sysmex XN and non-XN series (not our exact model) from a network of laboratories found sigma metrics consistently >4 with the exception of HCT, which had a number of <4 sigma occurrences.[4] On review of our sigma metrics, HCT also had the most <4 sigma occurrences. This was challenging to compare with other studies as HCT is measured on Sysmex analyzers and calculated on most other hematology analyzers.

5 | CONCLUSION

The results from this study show that we could evaluate the performance of the Sysmex XT-2000*i*V across a network of laboratories and achieved harmonization and optimization of procedures. We determined that the total error budget is indeed contained within ASVCP guidelines or internal expert opinion goals. This harmonization process demonstrated that sigma metrics are a useful monitor of performance across a network of laboratories capable of highlighting measurands that require attention (and specifically sigma metrics <5 warrants investigation and attention from Sysmex technical support). Keeping sigma metrics consistently above 5 ensures an efficient and effective quality management system. We intend to use sigma metrics <5 as the threshold for action to be taken to improve the performance for hematology in our laboratories. We intended to further evaluate the use of observed bias <3% and the failure to achieve the BV-based desirables—bias and the 0.33 x CV_I goal as indicators of unacceptable performance.

We saw continuous improvements in sigma metrics and most quality specifications during the three performance evaluation phases and have demonstrated that they are a good exercise in risk assessment and quality improvement. Further investigation is needed to determine whether applying more stringent QC rules based on the observed performance could be successful in improving performance.

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