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ORIGINAL ARTICLE

Repeat patient testing-quality control with canine samples shows promise as an alternative to commercial quality control material for a network of four Sysmex XT-2000*i*V hematology analyzers

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Abstract

Background: Repeat patient testing-quality control (RPT-QC) uses retained patient samples as an alternative to commercial quality control material (QCM). We elected to calculate and validate RPT-QC limits for red blood cell count (RBC), hemoglobin (HBG), hematocrit (HCT), and white blood cell count (WBC).

Objectives: (1) To validate RPT-QC across a network of four harmonized Sysmex XT-2000*i*V hematology analyzers and determine the total error that can be controlled with RPT-QC. (2) To generate quality control (QC) limits using the standard deviation (SD) of the duplicate measurement differences and determine a suitable simple QC rule with a probability of error detection >0.85 and probability of false rejection <0.05. (3) Monitor RPT-QC using sigma metrics as a performance indicator and (4) to challenge RPT-QC to ensure acceptable sensitivity.

Methods: Fresh adult canine EDTA samples with results within reference intervals were selected and run again on days 2, 3, and 4. QC limits were generated from the SD of the duplicate measurement differences. The QC limits were challenged using interventions designed to promote unstable system performance. The total error detectable by RPT-QC was determined using EZRULES 3 software.

Results: In all, 20-40 data points were needed for RPT-QC calculations and validated using 20 additional data points. The calculated limits differed among the network of analyzers. The total error that could be controlled was the same or better than that of the manufacturer's commercially available quality control material using the same analyzer for all measurands except hematocrit, which required a higher total error goal than that proposed by ASVCP guidelines to achieve an acceptable probability of error detection. The challenges designed to mimic unstable system performance were successfully detected as out-of-control QC.

Conclusions: The challenges for RPT-QC resulted in acceptable detection of potential unstable system performance. This initial study demonstrates that RPT-QC limits differ among the network of Sysmex XT-2000*i*V analyzers, indicating a requirement

to customize for the individual analyzer and laboratory conditions. RPT-QC could achieve ASVCP total allowable error goals for RBC, HGB, and WBC, but not for HCT. Sigma metrics were consistently >5.5 for RBC, HGB, and WBC, but not for HCT.

KEYWORDS
commercial quality control, harmonization, hematology, repeat patient testing-quality control, Sysmex

1 | INTRODUCTION

Quality control procedures for hematology analyzers are necessary to ensure that the quality of operations is maintained and that reliable results are produced.[1] The traditional method of ensuring quality controlled hematological data uses one or more commercial quality control material (QCM). Commercial QCM used in veterinary medicine is typically derived from human blood and synthetic/artificial components; therefore, may not comprise a commutable matrix for veterinary specimens. A preferable matrix would be from the animal origin, which includes components comparable to the patient throughput.[2] There is not an available supply of veterinary-specific commercial QCM for hematology; therefore, veterinary laboratories have little choice but to use a QCM with a potentially suboptimal matrix. Commercial QCM is a costly but necessary expense and has a relatively short shelf life.[3] Most importantly, it does not provide appropriate assurances as to how the veterinary specimens may behave on the analyzer and, therefore, is substandard in its application to a veterinary laboratory.

Repeat patient testing-quality control (RPT-QC) offers an alternative to commercial QCM by using retained patient samples to determine analytical stability. It relies on the principle that under specific storage conditions and time intervals, a labile EDTA specimen will deteriorate in a predictable way from the baseline measurement to the later repeated measurement of the same specimen. A repeated measurement exceeding thresholds of expected deterioration suggests instability within the analytical system. The concept of RPT-QC has been well documented in human medicine; however, its application has varied.[4–7] Recommendations for an RPT-QC protocol have been proposed,[8] in which a pilot set of data is gathered over a minimum of 20 days to generate control limits. These limits can then be used to determine whether RPT-QC results are "in-control" or "out-of-control." After this initial limit-derivation phase, a fresh RPT sample would be measured on Day 1 and again within a defined time interval and defined storage conditions. It has been recommended to use patient samples yielding within reference interval results as they represent a significant proportion of caseload and may avoid extreme variabilities between measurements.[9] The difference between original and repeated analyses can then be calculated and plotted similarly to other QC procedures.[7] There have been some positive reports in the literature[10–12] that indicate a good potential for application in veterinary laboratories.

This study addresses the following hypotheses.

1. That RPT-QC limits could be generated and validated across a network of four Sysmex XT-2000*i*V hematology analyzers.
2. That RPT-QC performance can achieve the ASVCP-recommended total allowable error (TE_a) goals using a simple QC rule, that is, 1-2.5 s or 1-3 s, a probability of error detection (P_{ed}) of ≥0.85, and a probability of false rejection (P_{fr}) of ≤0.05.
3. That Sigma metrics is a satisfactory monitoring tool for RPT-QC, and sigma metrics >6 (reflecting world class performance) can be achieved.
4. That deliberately created QC "challenges" mimic unstable performance that is detectable by RPT-QC.

2 | MATERIALS AND METHODS

2.1 | Analyzers and measurands

RPT-QC data from four Sysmex XT-2000*i*V analyzers (Sysmex Corporation, Kobe, Japan) were evaluated for the following measurands: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), and white blood cell count (WBC). These analyzers were previously optimized and harmonized[13] to ensure stable performance prior to this study. Analyzers were designated analyzer 1 (located in the first author's laboratory), 3, 4a, and 4b. The laboratory with analyzer 2 was unable to participate and is not included in this report. A single level of commercial QCM (level 2 - Normal e-CHECK (XE)-Hematology Control), which is composed of stabilized human RBCs, human WBCs, platelets, and nucleated RBC components in a preservative medium, was analyzed once per day at analyzer start-up, prior to analysis of patient samples, by a fully trained technician according to a standard operating procedure and was within accepted limits before assessing patient samples.[14] These measurands were chosen as those most useful in our network of laboratories for statistical QC.[14] Differential cell counts and platelet counts were considered to be more adequately assessed by nonstatistical QC by technician evaluations of blood smears.[14]

2.2 | RPT-QC limits generation

The process of RPT-QC data collection and calculations were conducted as recommended by Westgard.[9] Canine EDTA samples that were received by the laboratory for routine testing were selected by

a trained hematology technician for RPT-QC based on time of sampling (fresh/same-day submissions), surplus volume, and results that were within or close to reference intervals used by the laboratory. The day of accession was denoted "Day 1." The selected sample was refrigerated at 4-8°C and reanalyzed on days 2, 3, and 4 following acclimation to room temperature, thorough mixing, and rechecking for clots at each time point prior to analysis. The Day 1 minus Day 2 (or Day 1 – Day 2) change was used to define control limits that could be used for QC on successive days during the working week (Monday through Friday or Monday through Saturday). The Day 1 minus Day 3 (or Day 1 – Day 3) change was used to define control limits that could be used for QC on Mondays for those laboratories that worked Monday-Saturday. The Day 1 minus Day 4 (or Day 1 – Day 4) control limits were for QC on Mondays for those laboratories that worked Monday-Friday. This process was carried out across the network of four Sysmex analyzers, and the data were exported to Microsoft Excel (Microsoft Excel for Mac 2011, Version 14.7.7 [170905] Last update 14.7.7). Each analyzer accumulated 50-60 RPT-QC samples comprising a dataset of up to 40 samples for Day 1 – Day 2, Day 1 – Day 3, and Day 1 – Day 4 intervals to generate control limits.

A scatter plot of the differences of the repeated measurements was produced to identify outliers by visual examination. A maximum of three outliers were excluded from each control limit dataset following a visual review of a scatter plot of repeated measurements except for one dataset, Day 1 – Day 4 HGB, from which four were identified and removed. More complicated statistical evaluation for outliers was not undertaken to keep the procedure simple. If outliers were not readily apparent by visual examination, all data points were retained and included in the calculation of the RPT-QC limits. The mean of the differences for the repeated measurements for each time interval and the standard deviation of the duplicate (SD_{dup}) measurements were calculated for each time interval (Day 1 – Day 2, Day 1 – Day 3, Day 1 – Day 4). The coefficient of variation (CV) and the difference squared (Diff Sq) were calculated from the mean of the repeated measurements, as described previously.[9–11] See Figure 1 for example of initial calculations. The control limit range was calculated using the SD_{dup} and 1-2.5 s and 1-3 s control rules.

The following formula was used to calculate the SD_{dup}:

$$SDdup = \sqrt{\frac{\sum \text{difference}^2}{2n}}$$

where n is the number of observations.[15]

The control rule chosen for use was based on evaluation of the range of QC data compared to the width of the control limit interval (Figure 2) and whether or not validation was achieved by inclusion of at least 17 or more additional data points within the chosen control limits. If the QC data were not all included within the calculated limits or the data did not appear to adequately fill the calculated control limit interval, then an additional 10-20 QC data points were combined with the first dataset and control limits were recalculated using the 30-40 total QC data points, as described earlier (Figure 3).

	D1	D2	Aver D1,D2	Diff	Diff sq
1	15.8	15.8	15.8	0	0
2	15.3	15.3	15.3	0	0
3	11.7	11.7	11.7	0	0
4	15.4	15.6	15.5	-0.2	0.04
5	16.3	16.4	16.35	-0.1	0.01
6	13.8	13.7	13.75	0.1	0.01
7	14	14	14	0	0
8	14.9	15	14.95	-0.1	0.01
9	11.6	11.7	11.65	-0.1	0.01
10	15.2	15.2	15.2	0	0
11	18.4	18.4	18.4	0	0
12	17.6	17.7	17.65	-0.1	0.01
13	15.3	15.4	15.35	-0.1	0.01
14	14.3	14	14.15	0.3	0.09
15	15.3	15.5	15.4	-0.2	0.04
16	11.7	11.8	11.75	-0.1	0.01
17	16.3	16.6	16.45	-0.3	0.09
18	11.4	11.4	11.4	0	0
19	15.4	15.5	15.45	-0.1	0.01
20	14.9	14.9	14.9	0	0
averages	14.73	14.78	14.755	-0.05	0.017

FIGURE 1 An example of data calculations using 20 data points for hemoglobin Day 1 minus Day 2 (Day 1 – Day 2), showing Day 1 (D1) measurements, Day 2 (D2) measurements, the difference of Day 1 – Day 2 (Diff), an average of Day 1 and Day 2 measurements (Aver D1, D2), and the difference of the measurements squared (Diff sq).

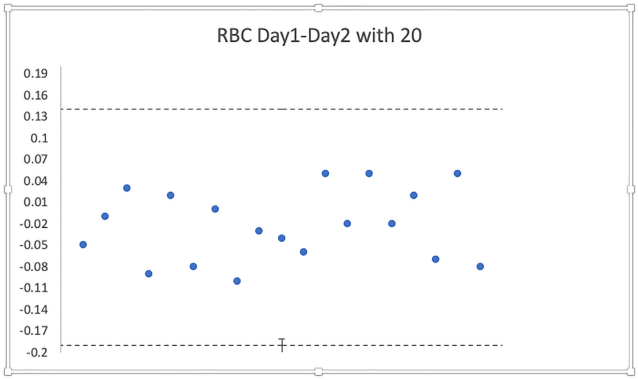

FIGURE 2 Initial scatter plot for RBC counts using 19 data points (one outlier removed) from the difference of Day 1 minus Day 2 (Day 1 – Day 2) samples used to calculate control limits for the 1-3 s rule. The dashed lines represent the upper and lower control limits (0.14 and −0.19, width 0.33). The graph shows the data does not adequately fill the control limits, which results in a poor sensitivity of detection of error.

2.3 | QC validation for calculated RPT-QC control limit interval

QC validation for the calculated RPT-QC control limits for each measurand, time period, and laboratory analyzer were undertaken using the following specifications (ASVCP recommendations for TE_a for hematology for allowable total error, number of QCM = n = 1, $P_{ed} \geq 0.85$, $P_{fr} \leq 0.05$, and simple rules used [1-2.5 s or 1-3 s]). The choice of $P_{ed} \geq 0.85$ was made because this is the minimum P_{ed} that can be achieved with a simple 1-3 s rule, and a single-level control result based on the models provided in the EZRules3 program. EZRules3 was then used to determine P_{ed} and P_{fr} that could be achieved using the recommended TE_a (ASVCP) for each of the

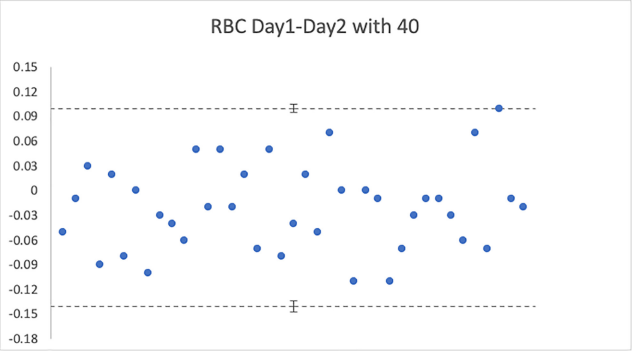

FIGURE 3 Subsequent scatter plot for RBCs using 39 data points (one outlier removed) from Day 1 minus Day 2 (Day 1 – Day 2) samples used to calculate control limits for the 1-3 s rule. The dashed lines represent the upper and lower control limits (0.1 and −0.14, width 0.24). The graph shows the data adequately fills the control limits, resulting in greater sensitivity for detecting out-of-control events with smaller width control limits.

measurands. EZRules3 was also used to determine the lowest TE_a that could be achieved using the control rules chosen based on a simple QC rule and CV of the mean difference for the measurand while achieving a $P_{ed} > 0.85$ and $P_{fr} < 0.05$.

2.4 | RPT-QC and sigma metrics

The sigma metric for each measurand and RPT-QC limit were calculated according to the following formula, using the ASVCP recommendations for allowable total error, the CV from each RPT-QC interval, and absolute bias = 0%:

$$\text{Sigma} = \left(\text{TE}_a\,\% - \text{absolute bias}\,\%\right) / \text{CV}\,\% = \text{TE}_a\,\% / \text{CV}\,\%$$

The SD, CV, mean difference, and control limit ranges for 1-3 s, and 1-2.5 s rules were calculated for each measurand and dataset. The CV was calculated using the mean of the averages (see Figure 1) of the Day 1 – Day 2, Day 1 – Day 3, or Day 1 – Day 4 results as follows:

$$\text{CV} = \left(\text{SD}_{dup} / \text{mean of averages of the Day 1 and repeated results}\right) \times 100$$

2.5 | RPT challenges

RPT-QC challenges were performed at a single laboratory (analyzer 1) over 1 week and were run as a single evaluation as per procedure for analyzing RPT-QC. Challenges were set up to create intentional sample alterations to test the control limits for RPT-QC. The sample alterations included using an aged sample past days Day 1-Day 4, freezing a sample, and a low-volume sample ("short collection" not coming up to the fill line) with excess EDTA, resulting in an elevated EDTA:blood ratio sample. Samples were also manipulated by spiking the sample with saline creating a hemodiluted sample. Hemoconcentrated samples were created by removing plasma. The addition of native canine lipids obtained from a lipemic serum sample to an EDTA blood sample created a lipemic sample.

3 | RESULTS

3.1 | RPT-QC limit generation

Tables 1–4 summarize the information pertinent for the generation of the RPT-QC limits for each measurand, time period, and analyzer. Upper and lower control limits were generated from the four Sysmex analyzers for WBC, RBC, HGB, and HCT using either 20, 30, or 40 QC data for Day 1 – Day 2, Day 1 – Day 3, and Day 1 – Day 4. Most datasets had at least one outlier; only four datasets were identified with no outliers. Forty data points were required for the generation of RPT-QC limits for most measurands. Only 10 of the 48 datasets across the measurands validated the initial calculations with 20 data points for either the 1-2.5 s or 1-3 s QC rules. Calculated RPT-QC limits that were obtained from calculations performed with 40 data points were subsequently validated with a further 20 data points.

Figure 2 illustrates the use of a scatter plot from the differences obtained for Day 1 – Day 2 for RBC using 19 data points (analyzer 1). One outlier was removed. Figure 3 illustrates a scatter plot for 39 data points as one outlier was removed for RBC Day 1 – Day 2 with RPT-QC limits indicated by dashed lines. The scatter plots show that the 40 data points more completely fill the range specified for the RPT-QC limits.

3.2 | QC Validation for RPT-QC intervals

Tables 1–4 summarize the QC validation and total allowable error that could be achieved for each measurand. ASVCP recommended TE_a quality goals of 10% total error were achievable across all measurands and time periods across all analyzers for RBC and HGB using the specified criteria of a simple rule, single quality control assessment, and P_{ed} and P_{fr} described earlier. Analyzer 4b could not achieve the ASVCP recommended quality goal for WBC for Day 1 – Day 2 and Day 1 – Day 3, though the other three analyzers could. HCT could not achieve ASVCP recommended TE_a across all analyzers and time periods.

3.3 | RPT-QC and sigma metrics

Sigma metrics were >6 for each analyzer for all measurands except for HCT, which was <3 sigma across all analyzers and measurands using the ASVCP recommended total allowable error quality goal of ±10%.

3.4 | RPT-QC challenges

The intentional sample alterations using saline-diluted and frozen samples were detected as out-of-control by the RPT-QC for all

TABLE 1 Repeat patient testing-quality control. The standard deviation (SD), coefficient of variation (CV), mean difference, control limits range, ASVCP-recommended total allowable error (TE_a) goal, lowest achievable total allowable error (TE_a), sigma metrics, and QC rule obtained for white blood cell counts (WBC).

Analyzer	1			3			4a			4b		
Day (time interval)	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4
SD	0.159	0.169	0.227	0.21	0.175	0.234	0.15	0.15	0.19	0.278	0.238	0.22
CV	1.29	1.51	1.85	2.04	1.76	2.47	1.69	1.75	2.12	2.88	2.73	2.35
Mean difference	−0.111	−0.0395	−0.556	−0.06	0.098	0.022	0.009	−0.071	−0.018	0.155	−0.214	0.094
Control limits range (+/−)	0.796	0.843	1.42	1.04	1.1	1.17	0.906	0.928	1.123	1.65	1.42	1.32
ASVCP TE_a (%)	15	15	15	15	15	15	15	15	15	15	15	15
Lowest achievable TE_a (%)	8	10	12	11	10	14	11	11	13	18	17	15
Sigma metric based on ASVCP recommended TE_a	11.63	9.93	8.11	7.35	8.52	6.07	8.88	8.57	7.11	6.25	6.23	6.47
QC rule	1-2.5 s	1-2.5 s	1-3 s	1-2.5 s	1-3 s	1-3 s	1-3 s	1-3 s	1-3 s	1-3 s	1-2.5 s	1-3 s

Abbreviations: ASVCP, American Society of Veterinary Clinical Pathology; CV, coefficient of variation; D1-D2, Day 1 – Day 2; D1-D3, Day 1 – Day 3; D1-D4, Day 1 – Day 4; quality control, QC; SD, standard deviation; TE_a, total allowable error.

TABLE 2 Repeat patient testing-quality control. The standard deviation (SD), coefficient of variation (CV), mean difference, control limits range, ASVCP recommended total allowable error (TE_a) goal, lowest achievable total allowable error (TE_a), sigma metrics, and QC rule obtained for red blood cell counts (RBC).

Analyzer	1			3			4a			4b		
Day (time interval)	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4
SD	0.039	0.042	0.062	0.061	0.104	0.084	0.033	0.049	0.039	0.082	0.076	0.065
CV	0.56	0.60	0.90	0.86	1.47	1.18	0.50	0.75	0.58	1.21	1.11	0.96
Mean difference	0.021	−0.033	−0.015	0.005	0.007	0.076	0.008	−0.013	−0.025	−0.056	−0.034	−0.017
Control limits range	0.240	0.21	0.37	0.36	0.52	0.5	0.199	0.297	0.233	0.412	0.454	0.325
ASVCP TE_a	10	10	10	10	10	10	10	10	10	10	10	10
Lowest achievable TE_a	4	4	6	5	8	7	3	5	4	8	7	6
Sigma metric based on ASVCP recommended TE_a	17.86	16.78	11.17	11.64	6.8	8.43	20.12	13.33	17.33	8.26	9.01	10.42
QC rule	1-3 s	1-2.5 s	1-3 s	1-3 s	1-2.5 s	1-3 s	1-3 s	1-3 s	1-3 s	1-2.5 s	1-3 s	1-2.5 s

Abbreviations: ASVCP, American Society of Veterinary Clinical Pathology; CV, coefficient of variation; D1-D2, Day 1 – Day 2; D1-D3, Day 1 – Day 3; D1-D4, Day 1 – Day 4; quality control, QC; TE_a, SD, standard deviation; total allowable error.

TABLE 3 Repeat patient testing-quality control. The standard deviation (SD); coefficient of variation (CV), mean difference, control limits range, ASVCP recommended total allowable error (TE_a) goal, lowest achievable total allowable error (TE_a), sigma metrics, and QC rule obtained for hemoglobin concentrations (HGB).

Analyzer	1			3			4a			4b		
Day (time interval)	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4
SD	0.085	0.49	0.083	0.081	0.061	0.63	0.134	0.152	0.168	0.117	0.127	0.164
CV	0.5	0.48	0.5	0.566	0.734	0.386	0.847	0.965	1.07	0.715	0.127	0.164
Mean difference	−0.039	−0.02	−0.003	−0.034	−0.029	0.042	−0.113	−0.108	−0.154	−0.089	−0.144	−0.127
Control limits range	0.5	0.48	0.5	0.45	0.36	0.36	0.804	0.76	0.84	0.701	0.635	0.82
ASVCP TE_a	10	10	10	10	10	10	10	10	10	10	10	10
Lowest achievable TEa	4	4	4	3	5	3	6	6	7	5	5	7
Sigma metric based on ASVCP recommended TEa	19.88	20.83	20	17.67	13.61	7.77	11.81	10.36	9.34	13.99	12.61	10
QC rule	1-3s	1-3s	1-3s	1-2.5s	1-3s	1-2.5s	1-3s	1-2.5s	1-2.5s	1-3s	1-2.5s	1-2.5s

Abbreviations: ASVCP, American Society of Veterinary Clinical Pathology; CV, coefficient of variation; D1-D2, Day 1 – Day 2; D1-D3, Day 1 – Day 3; D1-D4, Day 1 – Day 4; quality control, QC; SD, standard deviation; TE_a, total allowable error.

TABLE 4 Repeat patient testing-quality control. The standard deviation (SD), coefficient of variation (CV), mean difference, control limits range, ASVCP recommended total allowable error (TE_a) goal, lowest achievable total allowable error (TE_a), sigma metrics, and QC rule obtained for hematocrit (HCT).

Analyzer	1			3			4a			4b		
Day (time interval)	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4	D1-D2	D1-D3	D1-D4
SD	1.67	2.67	4.14	2.25	3.23	4.86	1.96	3.23	4.23	2.21	3	4.6
CV	3.47	5.41	8.24	4.47	6.21	8.34	3.94	6.37	8.23	4.34	5.84	8.78
Mean difference	−2.25	−3.98	−5.71	−3.15	−4.76	−6	−2.66	−4.42	−5.81	−2.89	−4.12	−6.22
Control limits range	8.37	13.33	20.7	11.28	16.12	15.81	9.89	16.15	21.15	11.04	14.98	22.99
ASVCP TE_a	10	10	10	10	10	10	10	10	10	10	10	10
Lowest achievable TE_a	22	34	50	25	35	45	25	39	50	27	37	54
Sigma metric based on ASVCP recommended TE_a	2.88	1.85	1.21	2.24	1.61	1.2	2.54	1.57	1.22	2.3	1.71	1.14
QC rule	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s	1-2.5s

Abbreviations: ASVCP, American Society of Veterinary Clinical Pathology; CV, coefficient of variation; D1-D2, Day 1 – Day 2; D1-D3, Day 1 – Day 3; D1-D4, Day 1 – Day 4; quality control, QC; SD, standard deviation; TE_a, total allowable error.

measurands. Spiking samples with native canine lipids, excessive EDTA, and incorrect storage were successful in causing errors in some but not all measurands. WBC was out-of-control with added lipid. RBC counts and HGB concentrations were out-of-control in the specimens with excessive EDTA. RBC, HGB, and HCT were out-of-control when the specimen was incorrectly stored (extended storage beyond 4 days).

4 | DISCUSSION

We found that RPT-QC limits could be generated using a single protocol across our network of laboratories using four Sysmex hematology analyzers. Additional information regarding the stability of specimens under defined storage and handling conditions is also possible from assessment of these data but beyond the scope of this study and article. The collection of RPT data across four Sysmex analyzers was the most challenging process of this study. The provision of a spreadsheet to technicians for entering data was essential for managing the RPT samples and data daily. Initial assessments across analyzers and time periods for RPT-QC demonstrated that more than 20 data points are often required to produce RPT-QC limits that will validate with a subsequent dataset of 20 results. This differed from prior recommendations to collect 20 data points[9] and prior studies that were able to validate limits determined using 20 data points.[10–12] Following the RPT-QC limit generation, the validation process required calculation of limits using 30-40 data points for more than half of measurands/time interval evaluations. We found that by only using 20 results, the data were either not included within the control limits or did not adequately fill the range, but when an additional 10-20 data points were used, then we saw a better distribution across the QC data range. Except for HCT, successful validation with 20 data points was more likely for Day 1 – Day 2 limits than Day 1 – Day 3 and Day 1 – Day 4. The calculations using 30-40 data points were more often required for Day 1 – Day 3 and Day 1 – Day 4 RPT-QC time intervals, likely due to more variability in the data due to increased aging. We found the use of scatter plots highly valuable to visualize the distribution of the data and for outlier identification. Intuitively you would think the SD of the duplicates would provide RPT-QC limits that were not too wide or too narrow. However, when assessed visually, some RPT-QC limits were too narrow for the dataset, and others appeared too wide, with the possibility of decreased sensitivity in error detection (Figure 2), which necessitated the use of additional data points to calculate the control limits.

As the sample aged, the trend was for the QC range to become wider, with higher SDs and CVs. This is not unexpected since more variation is expected with increased sample aging. However, it was interesting that the differences with increased aging were not uniformly similar within or across the laboratories. We found this particularly evident for HCT, where we saw the biggest increases in range, SD, and CV as the sample aged. Interestingly, in a previous study, we identified one analyzer performing suboptimally in comparison to the others in the network[13]; this was also reflected in the RPT-QC performance for that analyzer (analyzer 4b), demonstrating reduced analytical sensitivity.

The total error goals represented by ASVCP recommendations for hematology were achievable for the specified conditions ($P_{ed} \geq 0.85$, $P_{fr} \leq 0.05$, simple QC rule [1-2.5 s or 1-3 s], and $n = 1$) for RBC and HBG for all four analyzers and for three of the four analyzers for WBC. The ASVCP recommended TE_a goals could not be achieved for HCT on any of the analyzers with RPT-QC. This was not an unexpected finding as HCT is affected by MCV which has been previously found to increase with sample aging[10,11,14] and, therefore, was not included in this study. The inclusion of HCT was important despite the increased TE associated with differences used for RPT-QC. Although the total error that could be controlled with RPT-QC was unacceptable for interpretation of patient results, knowledge of the expected difference for the various time intervals was considered important for QC and optimizing our QC procedures for RPT-QC.

The sigma metrics using ASVCP TE_a goal for the RPT-QC for RBC and HGB are clearly reflective of world-class performance. Suboptimal sigma metrics (<3 sigma) using the ASVCP quality goal of 10% was seen for HCT for all time intervals and measurands for each analyzer; the recommended TE_a of 10% could not be achieved (see Table 4) and high CVs were seen. Analyzers 3 and 4b had lower sigma metrics compared with the other analyzers and were due to the high CVs generated. The lower sigma metrics for analyzer 4b likely reflect suboptimal performance and the need for servicing to determine if better performance can be obtained from this analyzer. It is interesting that analyzer 4b has consistently been identified with poor performance within the group based on traditional QC performance evaluation and QC validation.[13] This illustrates the fact that some analyzers may not perform as well as others. The fact that analyzers 4a and 4b were within the same laboratory, with the same environmental conditions, maintenance, reagents, control materials, and technicians, indicates that sources of variation other than those inherent to the analyzer itself were unlikely. This analyzer required more frequent servicing (up to 2 times the servicing normally expected) to maintain performance based on our internal criteria for performance monitoring (sigma metrics <5.5). The 5.5 sigma metric goal is considered useful for HCT as a basis for requiring service. For other measurands (RBC, HGB, WBC), a sigma metric <5.5 indicates a need for service.

The RPT-QC challenges were sufficient to demonstrate that specimens falling outside of our selection criteria (fresh, canine EDTA samples filled to the fill line of the collection tube) caused out-of-control events using the QC limits for the reference analyzer, demonstrating that the QC limits were sufficient to detect the induced changes in sample quality.

4.1 | Implications for our network of analyzers using RPT-QC

We can accommodate a 20% TE_a goal cut-off for WBC counts that is within reference intervals for dogs rather than the recommended TE_a

of 15% by the ASVCP Quality Assurance and Laboratory Standards Committee,[16] based on the expert opinions of pathologists involved in our Quality Education, Performance, and Implementation Group (QEPI, comprised five clinical pathologists and seven laboratory technicians, managers, and directors with a special interest in quality). The use of 20% as a quality goal for TE_a for hematology does not result in significant changes in the interpretation of results, as indicated by the average difference of WBCs between Day 1 – Day 2, Day 1 – Day 3, and Day 1 – Day 4 of 0.155, −0.214, and 0.094, respectively.

Continued monitoring to determine if all analyzers can consistently achieve 20% total allowable error quality goals for WBC with RPT-QC is needed. The lowest achievable TE_a for HCT ranged from 22% to 27% across the analyzers for Day 1 – Day 2, from 24% to 39% for Day 1 – Day 3, and between 45% and 54% for Day 1 – Day 4 compared with the ASVCP recommendation of 10% (Tables 1–4). The TE_a for HCT that could be achieved was considered acceptable for RPT-QC because RBC and HGB were also assessed and able to achieve their respective ASVCP total error goals. In addition, a further control measure for HCT using a spun PCV (considered the gold standard for evaluation of the erythron in veterinary medicine) is generated whenever HBG×3 differs from the automated HCT by five or more units.

An alternative of Day 1 – Day 4 could be used for measurand measurements at least 6 hours apart to use on Mondays for laboratories operating on a Monday-Friday schedule. However, further research will be required to determine acceptable RPT-QC limits for this option.

Two QC rules (1-2.5 s and 1-3 s) were assessed based on their ability to achieve acceptable P_{ed} (≥85%) and P_{fr} (≤5%) when $n = 1$. The preferred QC rule is 1-3 s, where one violation exceeding ±3 SDs from the mean deems the QC run a failure, this simple approach can be easily adopted by the laboratory and pathology personnel. We found not all measurands for each time interval could be validated using the 1-3 s rule, in fact only 45% could be validated. We have tailored RPT-QC monitoring spreadsheets to include both 1-3 s and 1-2.5 s QC rules specific to each analyzer; however, educating/training the user on the differences in QC rule violations is imperative for successful implementation.

Sigma metrics were employed as a quality performance indicator, where sigma >6 is indicative of world-class performance.[8] Our previous findings[13] showed that a sigma metric <5.5 using commercially available QCM indicated instability within the analytical system with a need for further investigations and troubleshooting procedures. Additional studies are needed to determine an acceptable sigma metric cut-off for monitoring the performance of RPT-QC. For the measurands at time intervals with high CVs and unacceptable TE_a compared with the reference analyzer, we found this was reflected in the sigma metric. For example, Day 1 – Day 2 and Day 1 – Day 3 WBC intervals for analyzer 4b had a sigma metric of 6.25 and 6.23, respectively, compared with the reference analyzer, where sigma metrics were 11.63 and 9.93, respectively. The poor performance of HCT was also reflected in the sigma metrics for all analyzers in the network, demonstrating the utility of sigma metrics as a performance indicator for RPT-QC.

5 | CONCLUSIONS

We found that RPT-QC limits could be generated for a network of Sysmex XT-2000*i*V hematology analyzers, but that the use of 40 data points was frequently required to achieve the subsequent validation of the RPT-QC by a dataset of 20 points. The TE_a goals provided by the ASVCP for hematology can be achieved for RBC and HBG. Alteration in acceptable TE_a for WBC measurand from 15% to 20% was considered acceptable based on internal expert opinion, while larger TE_a goals for HCT (ranging from 22% to 54%, depending on the time interval) were considered acceptable based on the acceptable TE_a performance for RBC and HBG. Sigma metrics provide a unitless measurement of performance capacity and, as demonstrated in previous studies using commercially available QCM, provide a reliable indication of suboptimal performance. Continued evaluation of RPT-QC in parallel with commercial QCM is needed to determine if an RPT-QC sigma metric cut-off of <5.5 for the various measurands indicates the need for analyzer service to maintain and improve analyzer performance. The challenges produced for RPT-QC to mimic unstable system performance demonstrated that RPT-QC was sensitive to these alterations. Continued monitoring in parallel with commercial QCM is required to determine if RPT-QC is reliable for daily internal quality control for hematology.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that there is no conflict of interest.

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