Diagnostic update

Absolute nucleated red blood cell (nRBC) quantification in dogs on the IDEXX inVue Dx Cellular Analyzer

Introduction

Nucleated red blood cells (nRBCs) are immature erythroid precursors that retain a nucleus. In healthy adult dogs and cats, circulating red blood cells (RBCs) are anucleate, and nRBCs are normally confined to the bone marrow or spleen. nRBCs are released into peripheral blood only under specific conditions. nRBCs may accompany a regenerative bone marrow response, best assessed by an increased reticulocyte count. In contrast, circulating nRBCs in the absence of concurrent reticulocytosis may indicate other underlying disorders, including bone marrow or splenic disease.[1]

nRBCs are a recognized marker of severe illness in dogs and cats, associated with disease severity and, in some conditions, increased mortality.[2–9] In dogs, this includes heat stroke, splenic neoplasia, regenerative anemias, critical illness, trauma, SIRS, and lead poisoning.[2–6] In cats, it includes critical illness, trauma, FeLV/FIV, and other systemic illness.[7–9] Although circulating nRBCs have been associated with a poorer prognosis, prognostic value varies by disease state.[10]

In veterinary medicine, nRBCs are conventionally reported per 100 white blood cells (WBCs) based on manual blood film review. However, because this metric is influenced by the WBC count, leukocytosis or leukopenia can substantially alter the relative nRBC value even when the absolute nRBC concentration remains unchanged (table 1). This may result in significant misinterpretation of the severity of nRBCs and can complicate serial assessment over time.

Parameter	Case 1	Case 2	Case 3	Case 4
WBC (K/µL)	2	5	10	30
nRBC (K/µL)	0.3	0.3	0.3	0.3
nRBC/100 WBC	15	6	3	1

Table 1. Relative nRBC/100 WBC shifting with various WBC count at a fixed absolute nRBC concentration. nRBC/100 WBC = (nRBC ÷ WBC) × 100, with the absolute nRBC concentration held constant at 0.3 K/µL. The 15-fold spread in the relative value reflects only the changing WBC count, not any change in erythroid precursor release.

Advances in automated hematology analyzers have led to the advent of reporting nRBCs as an absolute concentration (K/µL), independent of WBC fluctuations.[11]

Automated enumeration of nRBCs has been available on human hematology analyzers since the late 1990s, and in 2016, the International Council for Standardization in Hematology (ICSH) recommended reporting nRBCs as absolute concentrations in SI units ($\times 10^9$/L) rather than per 100 WBCs where technology and IT capability allow, noting that absolute counts are more clinically meaningful.[12]

In veterinary medicine, nRBCs are conventionally reported per 100 WBCs based on manual blood film review. Two limitations drove this convention: automated instruments could not yet report absolute nRBC concentrations and impedance-based CBC analyzers counted nRBCs as leukocytes, making manual WBC correction necessary. In contrast to impedance-only-based analyzers, laser-flow cytometry hematology analyzers, such as the ProCyte Dx™ Hematology Analyzer, less commonly encounter interference of nRBCs with the total WBC count. The Sysmex™ XN-V Automated Hematology Analyzer, an analyzer common to reference laboratories, takes a comparable approach with a dedicated fluorescence channel that enumerates nRBCs. The ProCyte Dx analyzer was developed and is manufactured in partnership with Sysmex to mirror reference-lab technology and quality at the point-of-care. Both analyzers flag samples in which nRBCs are suspected so that the finding is not silently absorbed into the WBC count. nRBC flags indicate the need for blood morphology assessment to confirm or (less commonly) update the total and/or differential WBC count.

The IDEXX inVue Dx™ Cellular Analyzer delivers comprehensive in-clinic hematology results in combination with a ProCyte One™ or ProCyte Dx hematology analyzer. When nRBCs are identified by cellular analysis on the IDEXX inVue Dx analyzer, they are quantified as an absolute count (K/µL), and the total and differential WBC counts are updated (or confirmed) to account for their rare contribution to WBC.

nRBCs and RBCs are enumerated by AI-driven image analysis, determining a proportion of nRBC/RBC. That ratio is then multiplied by the RBC concentration from the CBC to yield the reported absolute nRBC concentration in K/µL.

The analyzer delivers what previously required a blood film or reference laboratory CBC (detection, confirmation, quantification, and a corrected differential) with no manual correction at any step, putting automated absolute nRBC counts right at the point-of-care.

In part due to how nRBCs were traditionally reported, no reference interval has existed for the automated absolute number of nRBCs considered within normal limits (or clinically insignificant) in dogs. The IDEXX inVue Dx™ analyzer reports abnormal RBC morphologic changes and provides diagnostic considerations that address their potential clinical significance when its results are combined with the CBC results of the ProCyte One™ or ProCyte Dx™ hematology analyzer. Generally, in glass-slide evaluation, the finding of 3 or more nRBCs per 100 WBC (the upper reference interval established by IDEXX Reference Laboratories and corresponding to 0.3 K/µL when WBC count is 10 K/µL) has been suggested as a clinically abnormal finding.

Methods

Reference interval generation

Absolute nRBC results measured on the Sysmex™ XN-V analyzer from a healthy reference cohort of 93 dogs were used for nRBC reference interval estimation. These dogs, also used in a previous reference interval study, were 1–7 years old, with no concerns on history or physical examination, normal results on serum chemistry, total T_4, IDEXX SNAP™ 4Dx™ Plus Test results, and no medications except routine heartworm and parasite preventives.[13]

Because nonparametric reference interval estimation requires at least 120 individuals, and robust methods are not estimable when a large proportion of observations occur at the assay's lower reporting limit, the Sysmex XN-V analyzer reference interval was estimated using a parametric approach,[14,15] with the upper limit set at the 97.5th percentile and the lower limit fixed at 0.

Comparison of nRBC from the IDEXX inVue Dx Cellular Analyzer to Sysmex XN-V Automated Hematology Analyzer

nRBC detection by the IDEXX inVue Dx analyzer was compared to the Sysmex XN-V Automated Hematology Analyzer, previously validated for this use.[11] A separate 192-dog clinical cohort that spanned a wide range of nRBC concentrations provided paired results from the Sysmex XN-V and IDEXX inVue Dx analyzers for cross-platform comparison.

Bias between methods was estimated by Passing-Bablok regression[16] on these data, and Spearman's rank correlation was calculated. Sysmex XN-V analyzer reference intervals were then transferred to the IDEXX inVue Dx analyzer using the estimated regression coefficients. Confidence intervals were estimated nonparametrically by bootstrap resampling of the data.

Results

Reference interval

The Sysmex XN-V analyzer upper reference limit was 0.12 K/µL (0.09–0.16) (figure 1), translating to an estimated IDEXX inVue Dx analyzer upper reference limit of 0.10 K/µL (0.05–0.14). The IDEXX inVue Dx analyzer nRBC reporting threshold was set at 0.3 K/µL, corresponding to the 99.9th percentile of the fitted reference distribution. This threshold also approximates the commonly cited value of 3 nRBCs per 100 WBCs on manual blood film review when the total WBC count is 10 K/µL.

Comparison of nRBC from the IDEXX inVue Dx Cellular Analyzer to Sysmex XN-V Automated Hematology Analyzer

In the 192-dog cohort, Sysmex XN-V analyzer nRBC concentrations ranged from 0 to 40.3 K/µL, with strong correlation to IDEXX inVue Dx analyzer results across this range (Spearman rho = 0.878; 95% CI 0.825–0.919) (figure 2a). Passing-Bablok regression showed a slope of 0.884 (0.791–0.93) and intercept of -0.018 (-0.047–0), with IDEXX inVue Dx analyzer results trending slightly lower than Sysmex XN-V analyzer nRBC counts. Because most samples fell within the 0–1 K/µL range, a zoomed in view of this range is shown where the same agreement trend is evident (figure 2b).

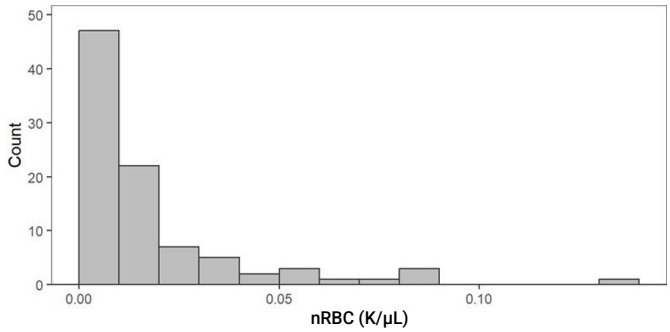

Figure 1. Distribution of nRBC concentrations from Sysmex XN-V analyzer for reference cohort of 93 dogs.

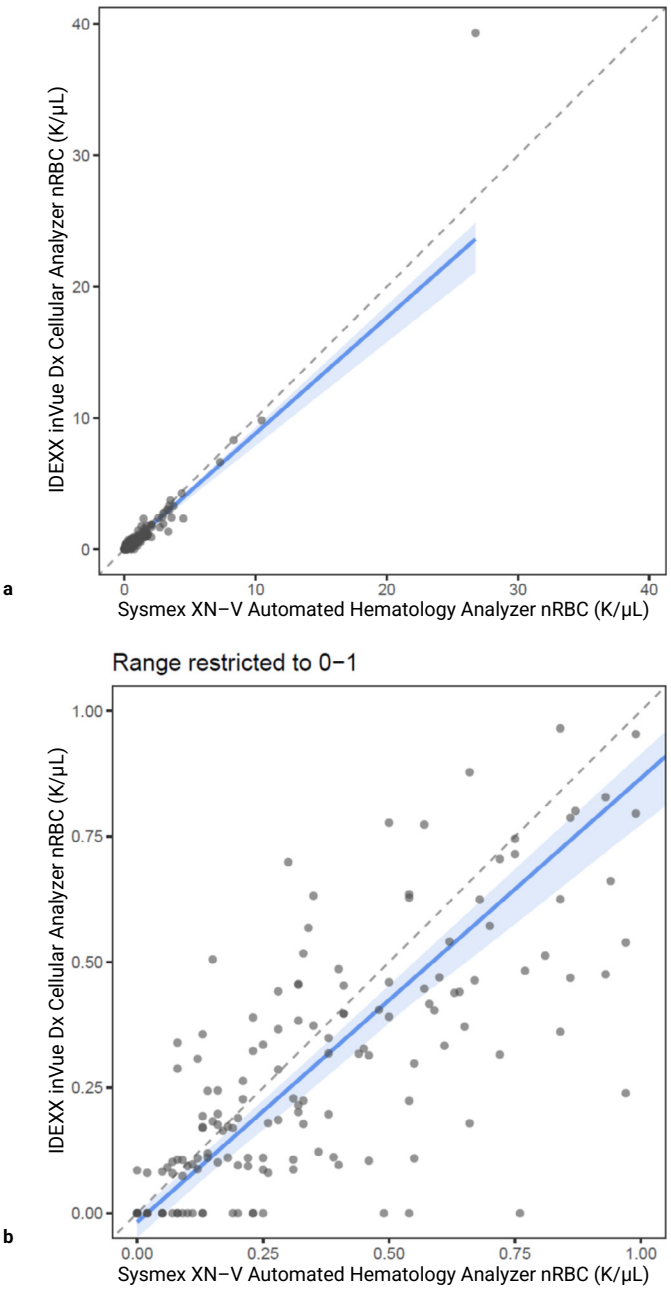

Figure 2. Sysmex™ XN-V analyzer vs. IDEXX inVue Dx™ analyzer nRBC concentration (n = 192), full range (a) and 0–1 K/µL inset (b).

Term	Estimate	95% CI
Intercept	-0.018	(-0.047, 0)
Slope	0.884	(0.791, 0.93)
Spearman rho	0.878	(0.825, 0.919)

Table 2. Passing–Bablok regression and Spearman correlation statistics for the comparison between Sysmex XN-V analyzer and IDEXX inVue Dx analyzer nRBC concentrations. CI, confidence interval; n = 192.

Discussion

This study establishes a reference interval and reporting threshold for absolute nRBC concentration on the IDEXX inVue Dx™ Cellular Analyzer. While further evaluation of clinical decision points for absolute nRBC counts is needed, absolute nRBC counts from the IDEXX inVue Dx analyzer provide a reproducible and consistent method for quantifying circulating nRBCs across disease states and in the context of reticulocyte counts. The reference interval presented here demonstrates that healthy adult dogs are expected to have little to no circulating nRBCs, so even a modest increase falls outside normal limits. A more conservative reporting threshold was set at 0.3 K/µL as this reflects three considerations: first, the reference interval was derived indirectly from nonanemic dogs without reticulocytosis and may not capture the full biologic variability of clinically healthy patients; second, at very low values of nRBCs, the concentration is calculated from a limited number of counted events and is therefore subject to counting imprecision at low levels; and third, because the IDEXX inVue Dx analyzer by design reports clinically actionable results and diagnostic considerations that prompt evaluation for underlying disease, the threshold favors specificity, so that a reported nRBC concentration reliably represents a true, potentially meaningful finding.

In the absence of established clinical decision points for absolute nRBC counts, nRBCs should be evaluated in parallel with reticulocytes and the patient's overall clinical picture. Although only reticulocyte counts should be used to determine if an anemia is regenerative, an increase in nRBCs (metarubricytosis) proportionate to reticulocytosis can reflect an appropriate regenerative response to hypoxia or erythropoietin drive, while disproportionate elevations suggest another pathology.

In nonregenerative anemias, nRBCs may indicate such states as bone marrow or splenic disease, precursor-directed immune-mediated anemia, chronic inflammatory disease (particularly renal disease), sepsis, neoplasia, or lead toxicity.

nRBCs are typically absent in nonanemic dogs; their presence may reflect marrow injury or marrow/splenic disease (e.g., heat stroke, splenic neoplasia, SIRS, lead toxicity, dyserythropoiesis, certain chemotherapeutics), though anecdotally low levels can be normal in some breeds (miniature schnauzer, dachshund). In the very least, serial monitoring will help to establish if the problem is persistent and worthy of more involved diagnostic tests.

Conclusions

nRBCs provide valuable insight into disease severity and underlying bone marrow or splenic pathology in dogs. Absolute reporting, unlike relative per-100-WBC values, is unaffected by changes in WBC count. The IDEXX inVue Dx™ Cellular Analyzer demonstrates strong agreement with the previously validated Sysmex™ XN-V Automated Hematology Analyzer, providing a superior way to evaluate circulating nRBCs to traditional blood films, a new ability to understand the true prevalence and significance of nRBCs, and the ability to monitor patient trends in nRBC counts directly.

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