DELTA TOTAL NUCLEATED CELLS ASSESSED VIA SYSMEX XT-2000iV AND SYSMEX XN-1000V ON FELINE EFFUSIONS: COMPARABLE DIAGNOSTIC ACCURACY FOR FELINE INFECTIOUS PERITONITIS

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Background: The Sysmex XT-2000iV delta total nucleated cell count (DTNC-XT), calculated based on the DIFF and BASO counts, is higher in feline infectious peritonitis (FIP) effusions than in any other disease.[1-3] The acidic reagent of the BASO channel induces clotting of high molecular weight proteins that are present in FIP effusions. The clots entrap the cells, leading in FIP effusions to a BASO count lower than the DIFF count, and subsequently a higher DTNC-XT.

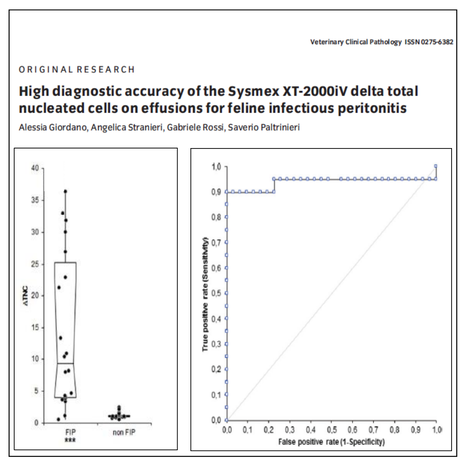

Figure 1*: results of the study about the utility of the DTNC-XT to diagnose FIP[2]*

The new Sysmex XN-1000V reagents used for cell counting in the channels named WNR and WDF differ from those of the corresponding Sysmex XT-2000iV channels (BASO and DIFF, respectively). The performances of DTNC-XN to diagnose FIP have not been investigated.

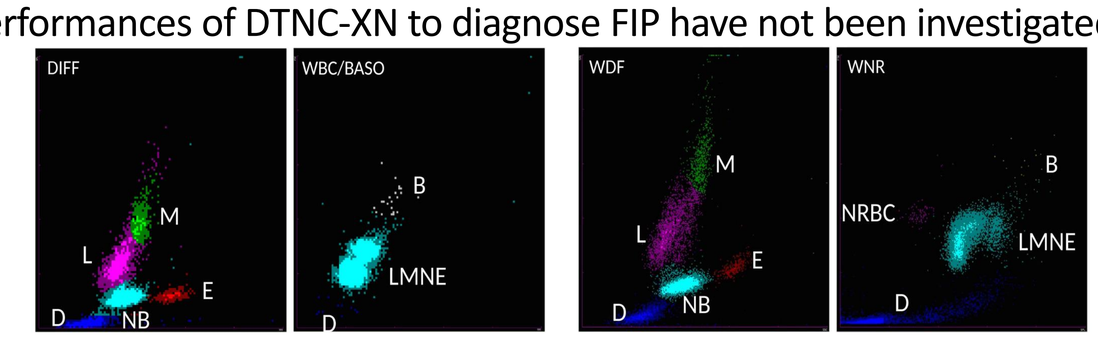

Figure 2: *Example of scattergrams of the Sysmex-XT 2000iV (left) and of the Sysmex XN-V (right). B=basophils; D=debris; E=eosinophils; L=lymphocytes; LMNE=all the WBC populations except basophils; M=monocytes; NB=neutrophils; NRBC=nucleated RBCs*

Objectives: 1) to compare the DTNC-XN with the DTNC-XT values; 2) to evaluate the ability of the DTNC-XN to diagnose FIP.

Methods: 36 feline effusions were analyzed with both instruments, irrespective of cytological findings and final diagnoses. The DIFF and the WDF, the BASO and the WNR, and the DTNC-XT and the DTNC-XV were compared to each other using a non-parametric t-test for paired samples (Wilcoxon signed rank test). The correlation was assessed using the Spearman test, while the agreement was assessed using the Passing & Bablok and the Bland Altman test. The concordance in detecting samples with DTNC-XT >1.7 (suggestive of FIP according to a previous study,[2] n=10) or >2.5 (consistent with FIP, according to a previous study, [2] n=9) was calculated in terms of Cohen's kappa coefficient. ROC curves were designed to assess the discriminating power of the DTNC-XN to identify samples that, according to the DTNC-XT, were suggestive or consistent with FIP.

Results: The results regarding the counts of the different channels and the DTNC of each instrument are summarized in table 1

BASO and WNR did not significantly differ (P=0.814), and were strongly correlated (P<0.001, rs = 0.978) (figure 3).

Despite a slight constant error (slope: 0.976; 95% CI: 0.851 to 1.008; intercept: 87.2; 95% CI: 17.1 to 118.8), no significant bias was found (P=0.395)

	Mean ± SD (Median) I-III IQR (min-max)
WBC-BASO Sysmex XT-2000iV	5,0 ± 5,9 (2,3) 0,5-8,2 (0,1-23,5)
WBC-WNR Sysmex XN-1000V	5,3 ± 6,4 (2,5) 0,4-9,6 (0,0-27,4)
WBC-DIFF Sysmex XT-2000iV	7,6 ± 8,2 (4,9) 1,5-10,2 (0,1-36,4)
WBC-WDF Sysmex XN-1000V	7,3 ± 7,8 (4,9) 1,6-9,7 (0,1-34,9)
Delta-TNC Sysmex XT-2000iV	3,92 ± 6,16 (1,14) 1,07-3,55 (0,66-28,98)
Delta-TNC Sysmex XN-1000V	8,04 ± 16,65 (1,02) 0,95-4,68 (0,65-65,8)

Table 1: *Cells counted by the two channels of each instrument (cells x 10^3/µL) and corresponding Delta*

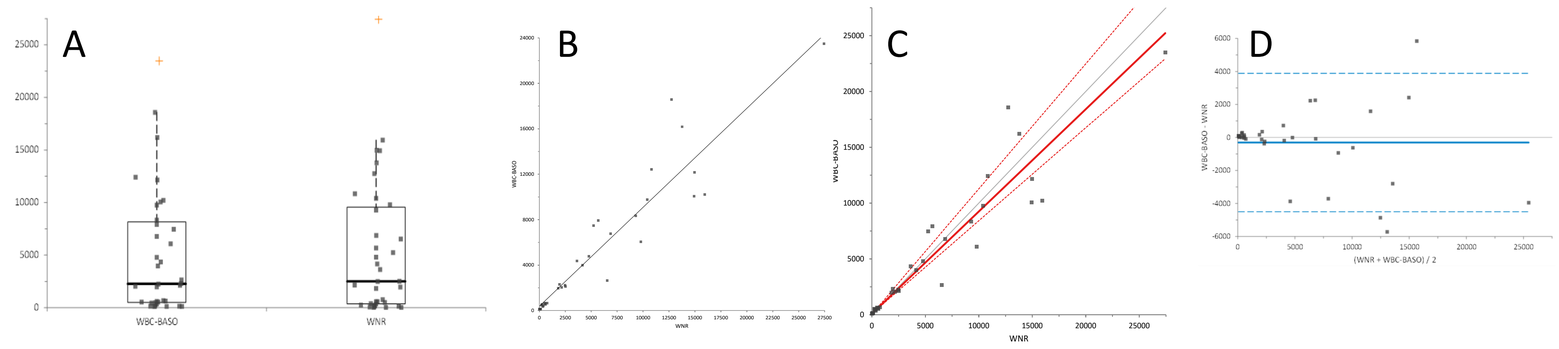

Figure 3: A*: comparison of the two cell counts (cell/µL). The boxes indicate the I-III interquartile range (IQR); horizontal lines indicate the median; vertical lines extend until the last value classified as «non outlier». The symbol «+» indicate the near ouliers (values higher than the III quartile + 1,5xIQR).* ***B****: Spearman test describing the correlation between the two counts;* ***C****: Passing&Bablok plot: the grey line is the identity line; the red line indicates the fit line; the dotted red lines indicate the 95% confidence intervals (CIs);* ***D****: Bland Altman plot: the grey line is the identity line; the blue line indicate the mean of the difference between the two counts; the dotted blue lines indicate the 95% CIs*

DIFF and WDF (P=0.346) did not significantly differ and were strongly correlated (P<0.001, rs = 0.981): constant or proportional errors (slope: 1.035, 95% CI: 0.965 to 1.081; intercept: 14.1, 95% CI: -94.7 to 94.4) or significant bias (P=0.394) were not found (figure 4).

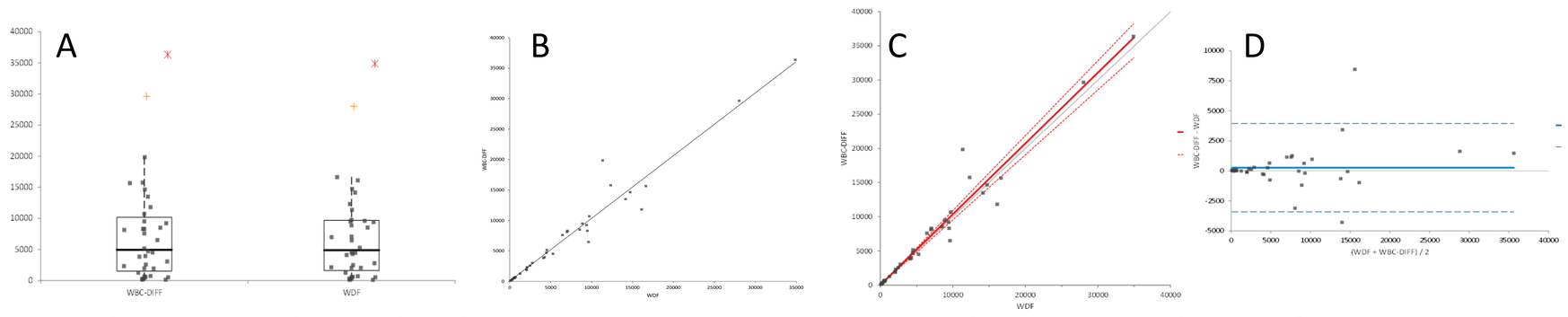

Figure 4: *A: comparison of the two cell counts (cell/µL);* ***B****: Spearman test describing the correlation between the two counts;* ***C****: Passing&Bablok* ***D****: Bland Altman plot. For the interpretation of the graphs see figure 3*

The two DTNCs did not significantly differ (P=0.850) and were strongly correlated (P<0.001; rs=0.706). A constant and a proportional error (slope: 1.878, 95% CI: 1.263 to 2.486; intercept: -1.062, 95% CI: -0.767 to 0.360) and a significant bias (P=0.039) were found (figure 5).

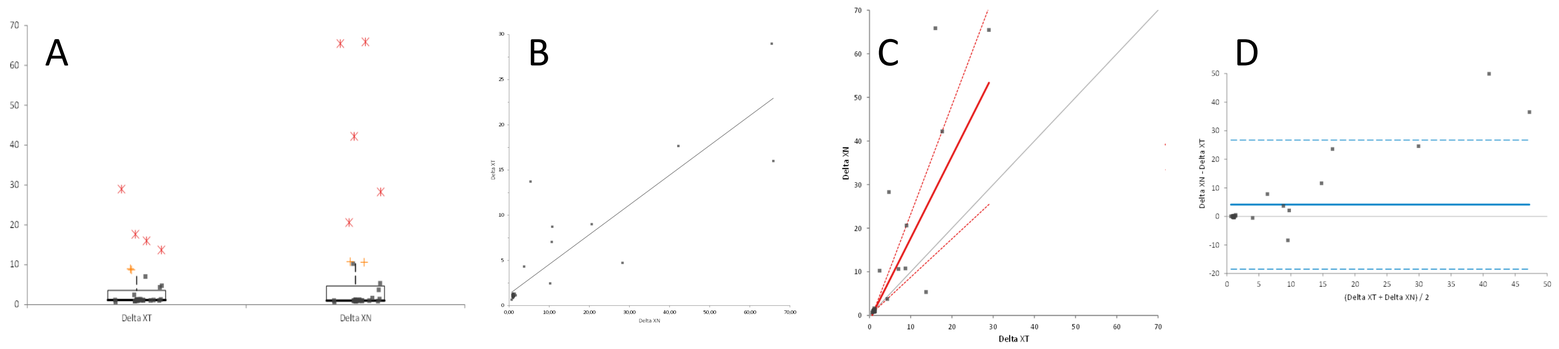

Figure 5: A*: comparison of the DTNCs (cell/µL);* ***B****: Spearman test describing the correlation between the two DTNCs;* ***C****: Passing&Bablok* ***D****: Bland Altman plot. For the interpretation of the graphs see figure 3*

The DTNC-XN correctly identified all the samples with DTNC-XT <1.7 or >1.7 and all but one samples with DTNC-XT >2.5 or <2.5 (table 2). The AUCs of ROC curves (Figure 6) were 1.000 (P<0.001) and 0.992 (P<0.001) at the thresholds of 1.7 and 2.5, with absolute specificity of DTNC-XN of >1.4 and >5.3

	XN >1.7		Total
XT<1.7	NEG	POS	
NEG	26	0	26
POS	0	10	10
Total	26	10	36

	XN >2.5		Total
XT<2.5	NEG	POS	
NEG	26	1	27
POS	0	9	9
Total	26	10	36

Table 2*: contingency tables displaying the concordance between the two instruments in classifying samples with DTNC-XT >1.7 or >2.5*

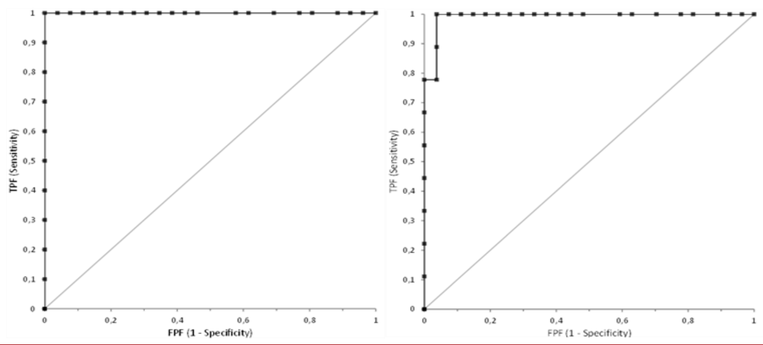

Figure 6: *ROC curves displaying the discriminating power of the DTNC-XN to identify samples with DTNC-XT >1.7 or >2.5. The grey line indicates the line of no discrimination*

Conclusion: Despite different raw values, the diagnostic accuracy for FIP of the DTNC-XN overlaps the diagnostic accuracy of the DTNC-XT.

References *[1]Pinto da Cunha N et al. (2009) Analytical validation of the Sysmex XT-2000iV for cell counts in canine and feline effusions and concordance with cytologic diagnosis. Vet Clin Pathol 38:230-241; [2]Giordano A et al. (2015) High diagnostic accuracy of the Sysmex XT-2000iV delta total nucleated cells on effusions for feline infectious peritonitis. Vet Clin Pathol 44:295-302; [3]Stranieri A et al. (2017) Diagnosing feline infectious peritonitis using the Sysmex XT-2000iV based on frozen supernatants from cavitary effusions. J Vet Diagn Invest. 29:321-324.*