Beta site evaluation of the new Pentra XLR and reticulocyte comparison with XE-5000 and the BD Retic-Count kit on FACSCanto II.

Jeroen MALFAIT[1], Melanie VANTIEGHEM[1], Stephane ROUGALE[2], Majda MAYEMI SAIFI[2], Marlène REYNAUD[2] and Veronique STOVE[1]

[1] Department of Laboratory Medicine, Ghent University Hospital, Belgium; [2] Horiba Medical, Montpellier, France

Background

The Pentra XLR is a new generation of Pentra analytic systems, able to perform the cell blood count (CBC) , a 5-part leukocyte differential (5 DIFF) analysis and a reticulocyte count.

The CBC and 5 DIFF are based on impedance, cytochemistry and the measurement of light absorbance, using the double-hydrodynamic sequential system (or DHSS*) technology. On the Pentra XLR, the flow cytometric reticulocyte analysis (RET) is performed by use of the thiazole orange dye. Young, immature reticulocytes are brightly fluorescent while maturing reticulocytes show an intermediate or low fluorescence intensity. Automated counting of reticulocytes has increased the accuracy and precision compared with traditional manual counts and provides the ability to reliably measure new parameters of RET maturation, such as immature reticulocyte fraction (IRF) and measurement of the reticulocyte hemoglobin content.

The aim of the present evaluation is to determine the analytical performance of the new Pentra XLR blood cell analyser. The results provided by this instrument were compared with those obtained by our routine hematology analyser. Moreover, RET results were compared with a reference method on a three laser flow cytometer.

** (HORIBA Medical Patent)*

Materials and methods

The study consisted of a performance qualification.

Blood samples

Blood samples (K2-EDTA) from our daily routine were used for CBC + DIFF (n =135) and RET (n=213) analysis. The samples were selected to have 50% as normal and 50% with pathologies, based on the expert rules of our routine hematology analyser.

Performance qualification

1- Reproducibility: this protocol follows the CLSI EP5-A2 guideline. The reproducibility is done on 3 control levels: low, normal and high with ABX Difftrol for DIFF mode and ABX Minotrol Retic for RET mode, with 4 runs/day/level.

2- Repeatability: within-run imprecision was evaluated based on 12 consecutive measurements on 4 samples that were assayed in RET mode. The test was performed on normal blood.

3- Accuracy / method comparison: coefficients of determination and Passing and Bablok regression analysis were used to evaluate agreement of Pentra XLR with the XE-5000 analyzer (Sysmex) for CBC+DIFF+RET analysis, and the BD Retic-Count™ kit on the BD FACSCanto II flow cytometer (Beckton Dickinson) for RET analysis.

RESULTS OF CBC + 5 DIFF

- Imprecision reproducibility

Run-to-run imprecision results are shown in Table 1 for CBC+DIFF, and Table 3 for RET parameters. All results were within the specifications established by the manufacturer.

		CBC count					5 DIFF count				
		WBC 10^9/L	RBC 10^{12}/L	HGB g/dl	HCT %	PLT 10^9/L	LYM 10^9/L	MON 10^9/L	NEU 10^9/L	EOS 10^9/L	BAS 10^9/L
Low	Mean value	2.33	2.44	6.56	19.80	66	0.69	0.04	1.35	0.18	0.07
	Obtained CV %	2.74	2.01	1.79	2.56	6.18	7.01	28.10	3.91	23.36	5.77
	Expected CV %	5.00	3.00	2.50	5.00	15.00	8.00	40.00	8.00	25.00	8.00
Normal	Mean value	7.08	4.75	13.63	38.80	256	2.22	0.19	4.05	0.37	0.24
	Obtained CV %	2.00	2.13	1.54	2.39	4.01	4.07	9.79	2.72	13.02	3.67
	Expected CV %	4.00	2.50	2.00	4.00	10.00	8.00	20.00	6.00	15.00	8.00
High	Mean value	16.66	5.31	16.58	46.53	521	2.72	0.48	11.77	0.97	0.72
	Obtained CV %	1.68	1.84	1.36	2.19	2.34	3.49	11.06	1.98	7.61	2.54
	Expected CV %	3.00	2.50	1.80	3.00	7.00	8.00	15.00	4.00	10.00	8.00

Table 1: Reproducibility of complete blood cell count (CBC), 5 DIFF count on new Pentra XLR analyzer.

- Correlation DIFF mode

The comparison between the Pentra XLR and XE-5000 was performed on 135 samples. A high degree of agreement between the 2 methods Pentra XLR and XE-5000 was observed, as estimated by the coefficient of determination regarding RBC, WBC, HCT, HGB, LYM and PLT , $R^2 \geq 0.95$ (Figures 1-4).

Fig 1

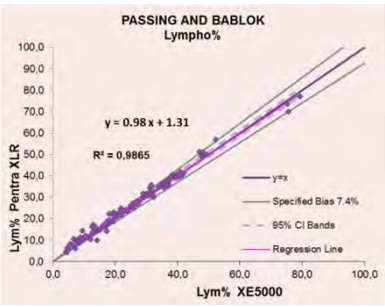


Fig 2

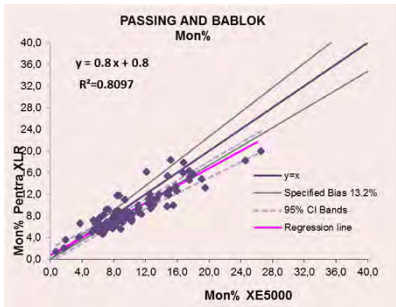


Fig 3

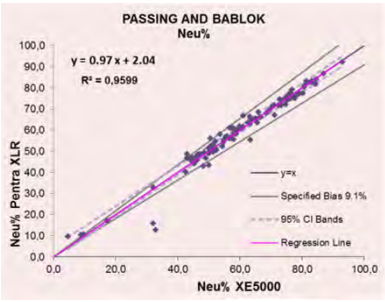
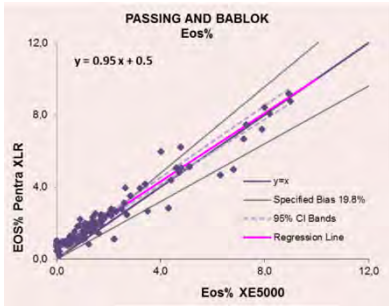


Fig 4



RESULTS OF RET

- Imprecision repeatability

The repeatability imprecision was performed for reticulocytes parameters on 4 samples. The results, shown in Table 2, were within the specifications stated by the manufacturer (RET% CV<12% et RET # CV <20%).

	RET %	RET #
CV %	5.41	5.33
SD	0,06 %	0,003 10^{12}/L
Mean	1.19 %	0.06 10^{12}/L

Table 2: Repeatability of reticulocyte count on new Pentra XLR analyzer. Results are from 1 sample and are representative of all samples.

- Imprecision reproducibility

	Reticulocyte count				
		RET %	RET L %	RET M %	RET H %
Level 1	Mean value	1.42	65.56	24	10,9
	Obtained CV %	8.69	5.14	14.27	25.81
	Expected CV %	18	11.00	24.00	40.00
Level 2	Mean value	4.59	47.04	36	16.92
	Obtained CV %	4.97	6.72	6.20	18.10
	Expected CV %	10.00	15.00	18.00	40.00
Level 3	Mean value	10.22	41.05	39	19.55
	Obtained CV %	3.29	9.01	4.54	17.83
	Expected CV %	8.00	18.00	17.00	40.00

Table 3: Reproducibility of reticulocytes count on new Pentra XLR analyzer.
RET L, M, H :RET Low, Middle and High degree of fluorescence (depending on maturation)

- Correlation RET mode

The method comparison between the Pentra XLR, XE-5000 and BD FACScanto II was performed for the RET parameters on 213 samples. As seen in Figures 5 to 7, the obtained correlation was good, especially between Pentra XLR and cytometry.

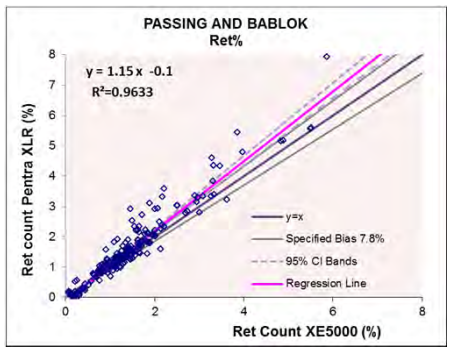


Fig 5

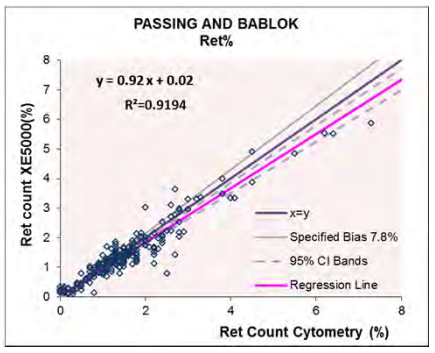
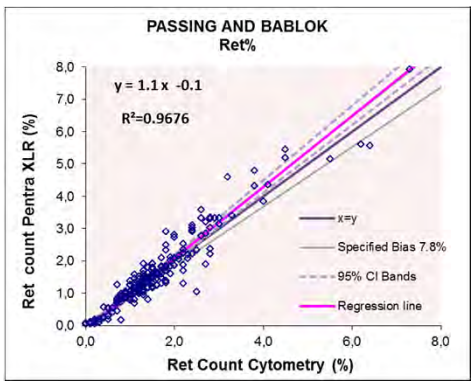


Fig 6

Fig 7



Conclusion

Repeatability and reproducibility were acceptable and within the specifications of the company. Method comparison between Pentra XLR and flow cytometry showed a good correlation with 5,0% bias between both methods. In conclusion, based on this evaluation, we can conclude that the Pentra XLR is showing a highly acceptable operational performance and is therefore suitable for the market.