Performance Validation of HORIBA Medical Yumizen H2500 Blood Cell Counter, Analytical Component for HORIBA Evolutive Laboratory Organisation (HELO*) Solution, Compared to DXH 800

Gilles Bonicelli[1], Philippe Halfon[1], Cécile Chabert[2], Elise Darrambide[2], Jean-Remy Gougaud[2], Franck Seguy[3], Bruce H Davis[3]
[1]Laboratoire Alphabio, Marseille , France, [2]Clinical Quality Dept. and [2]International Marketing, HORIBA Medical, Montpellier, France and [3]Bruce H Davis MD, Inc, Clifton, Maine, USA5

Introduction :

HORIBA Medical has released a new laboratory automation system for hematology testing, the HORIBA Evolutive Laboratory Organization (HELO*) system, which includes a new high throughput blood cell counters that offers CBC/DIFF parameters, including routine nucleated RBC counts, fluorescence based reticulocyte analysis and optical extinction platelet counts and body fluid (BF) counting. Initial validation studies were performed to confirm performance specifications on precision, LOQ, linearity, precision and method comparisons to other blood counter models and microscopic leukocyte differential counts, which are reported here. The cell differential matrix is based on the patented HORIBA Double Hydrodynamic Sequential System «DHSS» flow cytometric design.
Throughout the performances evaluation, we were able to collect a large number of clinical cases that were the subject of a dedicated study.

Methods:

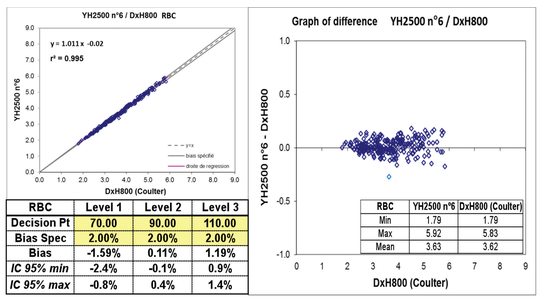
273 Fresh blood samples collected in K2 or K3 EDTA from patients and healthy controls were analyzed on a Yumizen H2500 instruments with Yumizen P8000 middleware for method comparison to Beckman Coulter DXH800 instruments. Method comparison and bias estimation were performed according to CLSI EP09-A3. Statistical analysis was performed using Passing Bablok regression fit with Pearson's and Bland Altman's difference plots. Precision, linearity and limit of detection were determined according to CLSI and ICSH guidelines.

Results:

Precision in the low, normal and high ranges of all parameters was determined to be below the manufacturing specifications for CV, including WBC <2%, RBC < 2%, HGB <1%, HCT <2%, PLT <5% (<10% below 30 x $10^{\wedge 9}$/L). Linearity was confirmed for platelet counts between <10 – 5000 x $10^{\wedge 9}$/L, for RBC counts 0.22 – 8.95 x $10^{\wedge 12}$/L, for HGB 0.6 – 24.5 G/L, for HCT 1.8 – 69.7%, for WBC 0.3 – 406 x $10^{\wedge 9}$/L and for reticulocytes 0.043 – 1.244 x 10^9/L). The correlation of the DXH - 8000 and Yumizen H2500 models exceeded 0.98 for the parameters of WBC, HBG, HCT, and PLT count and exceeded 0.90 for MPV, RBC and reticulocytes with samples covering the full analytical measurement range. Platelet counts by impedance and optical extinction methods correlated at levels also >0.95.

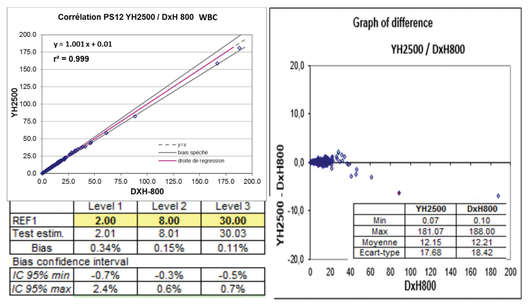
Conclusions:

The Yumizen H2500 instrument is safe, as effective, and performs as well or better than the BCI DXH-800 for all the usual CBC/DIFF parameters. The novel optical extinction platelet count performs as well or better than impedance counts. Ergometric features of the instrument were identified by the naïve users as those functions and custom flag settings, designed to be done within the Yumizen P8000 middleware module. The body fluids analytical validation will have to be evaluated on a larger statistical series (Fig 6).



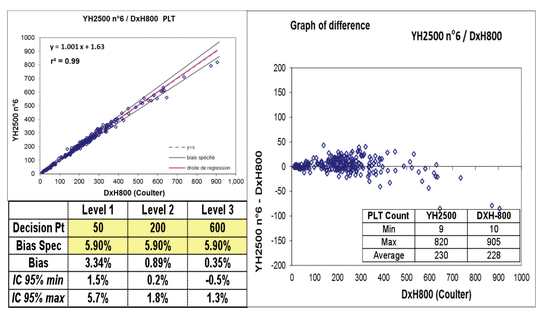
RBC	Level 1	Level 2	Level 3
Decision Pt	70.00	90.00	110.00
Bias Spec	2.00%	2.00%	2.00%
Bias	-1.59%	0.11%	1.19%
IC 95% min	-2.4%	-0.1%	0.9%
IC 95% max	-0.8%	0.4%	1.4%

RBC	YH2500 n°6	DxH800 (Coulter)
Min	1.79	1.79
Max	5.92	5.83
Mean	3.63	3.62



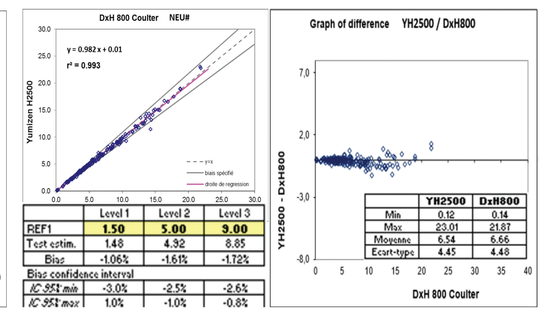
	Level 1	Level 2	Level 3
REF1	2.00	8.00	30.00
Test estim.	2.01	8.01	30.03
Bias	0.34%	0.15%	0.11%
Bias confidence interval			
IC 95% min	-0.7%	-0.3%	-0.5%
IC 95% max	2.4%	0.6%	0.7%

	YH2500	DxH800
Min	0.07	0.10
Max	181.07	188.00
Moyenne	12.15	12.21
Ecart-type	17.68	18.42



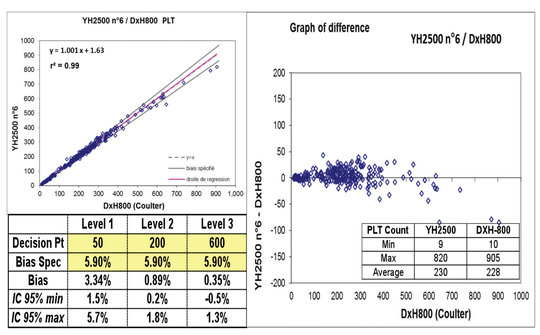
	Level 1	Level 2	Level 3
Decision Pt	50	200	600
Bias Spec	5.90%	5.90%	5.90%
Bias	3.34%	0.89%	0.35%
IC 95% min	1.5%	0.2%	-0.5%
IC 95% max	5.7%	1.8%	1.3%

PLT Count	YH2500	DXH-800
Min	9	10
Max	820	905
Average	230	228



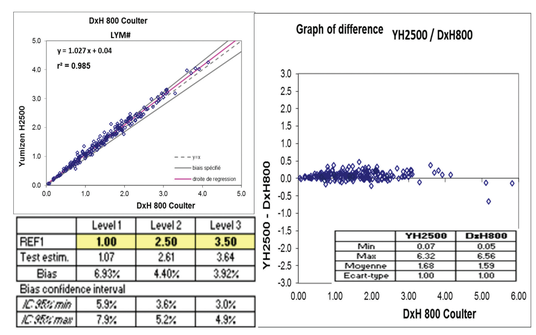
	Level 1	Level 2	Level 3
REF1	1.50	5.00	9.00
Test estim.	1.48	4.32	8.85
Bias	-1.06%	-1.61%	-1.72%
Bias confidence interval			
IC 95% min	-3.0%	-2.5%	-2.6%
IC 95% max	1.0%	-1.0%	-0.8%

	YH2500	DxH800
Min	0.12	0.14
Max	23.01	21.87
Moyenne	6.54	6.66
Ecart-type	4.45	4.48



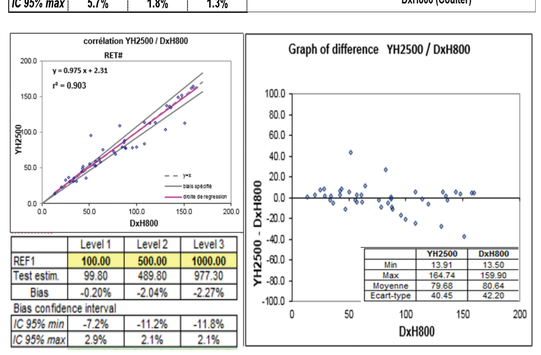
	Level 1	Level 2	Level 3
Decision Pt	50	200	600
Bias Spec	5.90%	5.90%	5.90%
Bias	3.34%	0.89%	0.35%
IC 95% min	1.5%	0.2%	-0.5%
IC 95% max	5.7%	1.8%	1.3%

PLT Count	YH2500	DXH-800
Min	9	10
Max	820	905
Average	230	228



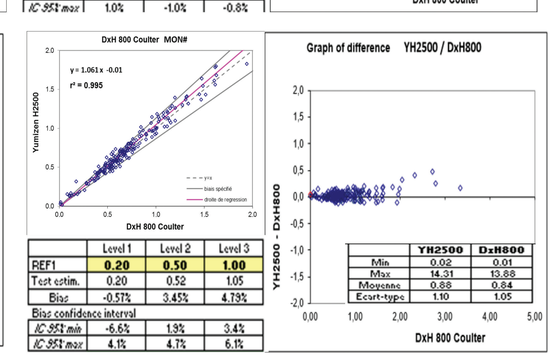
	Level 1	Level 2	Level 3
REF1	1.00	2.50	3.50
Test estim.	1.07	2.61	3.64
Bias	6.93%	4.40%	3.92%
Bias confidence interval			
IC 95% min	5.9%	3.6%	3.0%
IC 95% max	7.9%	5.2%	4.9%

	YH2500	DxH800
Min	0.07	0.05
Max	6.32	6.56
Moyenne	1.68	1.59
Ecart-type	1.00	1.00



	Level 1	Level 2	Level 3
REF1	100.00	500.00	1000.00
Test estim.	99.80	489.80	977.30
Bias	-0.20%	-2.04%	-2.27%
Bias confidence interval			
IC 95% min	-7.2%	-11.2%	-11.8%
IC 95% max	2.9%	2.1%	2.1%

	YH2500	DxH800
Min	13.91	13.50
Max	164.74	159.90
Moyenne	79.68	80.64
Ecart-type	40.45	42.20



	Level 1	Level 2	Level 3
REF1	0.20	0.50	1.00
Test estim.	0.20	0.52	1.05
Bias	-0.51%	3.45%	4.73%
Bias confidence interval			
IC 95% min	-6.6%	1.3%	3.4%
IC 95% max	4.1%	4.7%	6.1%

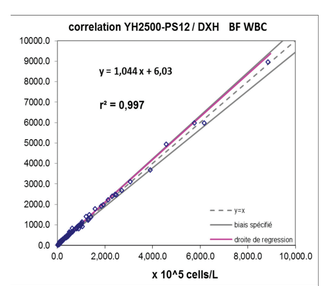
	YH2500	DxH800
Min	0.02	0.01
Max	14.31	13.88
Moyenne	0.88	0.84
Ecart-type	1.10	1.05

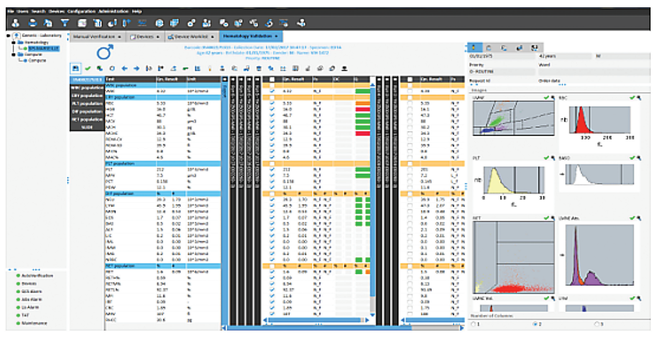
Carry-over Specimen Values		
Parameters	**Hight target value**	**Low Target value**
Whole Blood		
WBC (10^9/L)	**> 50**	**> 0.3 and < 3**
RBC (10^12/L)	**> 6,2**	**> 0.5 and < 1.5**
HB (g/L)	**> 200**	**> 20 and < 50**
RET (10^9/L)	**> 300**	**< 30**
PLT (10^9/L)	**> 800**	**> 10 and < 30**

Table 1. carry-over specimen values determined for YH2500.

Parameters	LoQ	LoQ Claimed	Linearity
WBC	0.1 x 10^3/µL	0.2 x 10^3 µL	0.20 – 350 x 10^3 /µL
RBC	0.22 x 10^6/µL	0.22 x 10^6 µL	0.24 – 8.81 x 10^3 /µL
Hgb	0.6 g/dL	0.6 g/dL	0.6 – 24.5 g/dL
HCT	1.90%	2.00%	2.0 – 68.8 %
PLT-i	4 x 10^3/µL	6 x 10^3/µL	6 – 5019 x 10^3 /µL
PLT-ox	12 x 10^3/µL	12 x 10^3/µL	12 – 3446 x 10^3 /µL
RET%	0.30%	0.30%	0.3 – 35.8 %
RET#	0.01 x 10^6/µL	0.01 x 10^6/µL	0.05 – 1.24 x 10^6 /µL

Table 2. Linearity and limits of quantification (LOQ) determined for YH2500.





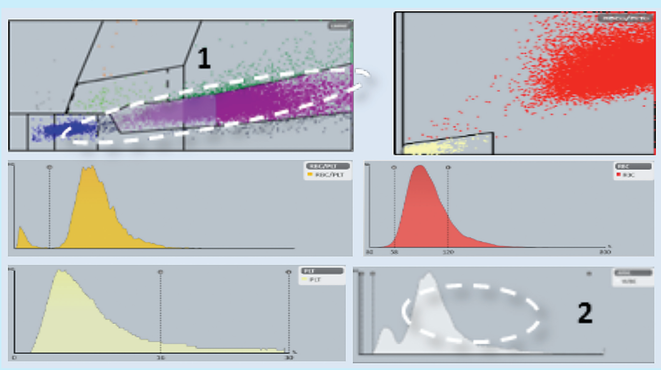
Picture 1. Results screen of Yumizen P8000 middleware dedicated for HELO* Solution

Clinical cases study:

is to assist all users to rapidly familiarize the laboratory scientists and physicians with the Yumizen H1500 and Yumizen H2500 data display. The key to optimal use of any cytometer or blood counter is the subtle use of details provided by careful review of the histograms and data matrix displays.

Pathology	Expected	Achieved
RBC Double population		
Patients transfused	5	5
Anemias		
Megaloblastic anemia	3	2
Thalassemia	6	6
Drepanocytose (Sickle cell disease)	3	1
Iron deficiency	10	10
Myelodysplasia	5	5
Erythroblastic anemia (nRBCs presence)	3	3
Sideroblastic anemia	2	0
Polycythemia vera	3	2
Post-splenectomy state or body Howell Jolly	3	0
Samples with blood clots and platelet aggregates	5	5
Chronic Leukemia		
CLL	10	10
CML	5	2
CMML	2	1
Acute Leukemia		
ALL	4	2
AML	6	11
Platelet Disorders		
ITP - Purpura thrombopenic idiopathic	3	1
TTP - Thrombotic Thrombocytopenic Purpura	2	1
Thrombocytosis	10	10
Myelodysplasia	5	5
Post-chemotherapy	14	15
Multiple myeloma	5	1
Multiple myeloma (plasmatic cells)	2	0
Reactive leucocytosis	10	10
Reactive lymphocytosis	5	5
Monocytose	3	3
Eosinophilia (>10% eosinophils)	5	4
Infections (viral, malaria, bacterial, HIV)	>=2	>2/1>2/2

Acute Myeloid leukemia M5



On the DIFF scattergram we observe the presence of immature monocytic population. The population is essentially composed of all step of monocytes maturation between the monoblasts, promonocytes and monocytes. The monoid population is perfectly defined in the DIFF scattergram in the dedicated area. We observe also the severe neutropenia.
On the WBC histogram, we observe the important ratio of the monocyte cells versus the lymphocyte or granular cells.

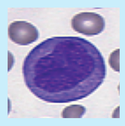
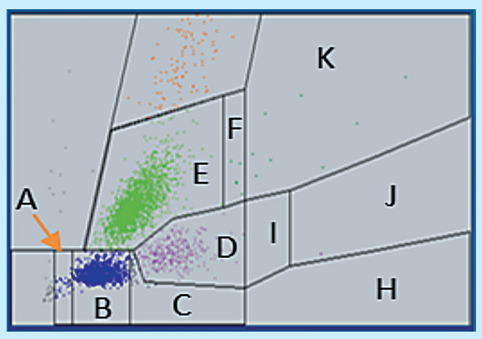


Fig 6 : Body Fluids Counts, Analysis of 123 various serous, CSF and synovial specimens

White cell matrix (left panel) for the cell differential counts for YH1500 and YH2500 display the populations of NRBC (A), Lymphocytes (B), "atypical" lymphocytes (C), monocytes (D), neutrophils (E) including "left-shifted" neutrophils (F), eosinophils (G), immature lymphocytes (H), immature monocytes (J) including "left-shifted" monocytes (I) and immature myeloid cells (K).



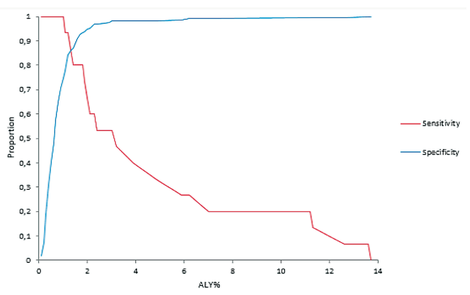
A threshold at 2% ALY on YUMIZEN H2500 provides 92 % of correlation with DxH relative sensitivity at 93% and relative specificity at 73%.

Statut DxH	Statut YH2500 LIC or ALY>2% Neg YH2500	Pos YH2500	Total
Neg DxH800	167	36	203
Pos DxH800	5	15	20
Total	**172**	**51**	**223**

statut DxH LYA	1	0	Total
	15	208	223

	AUC	95% CI		SE
ALY%	0,945	0,906	to 0,985	0,0201

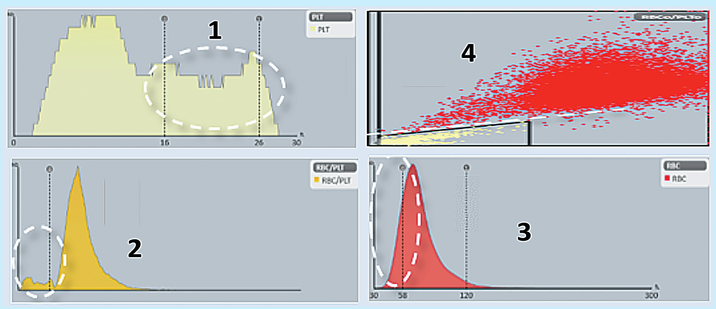
Decision Threshold



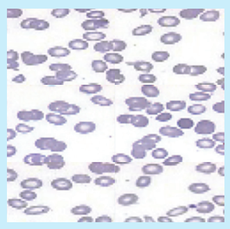
Cut-Off = 2%	Statut YH2500 (		
Statut dxH	Négatif YH2500	Positif YH2500	Total
Négatif DxH	195	13	208
Positif DxH	4	11	15
Total	199	24	223

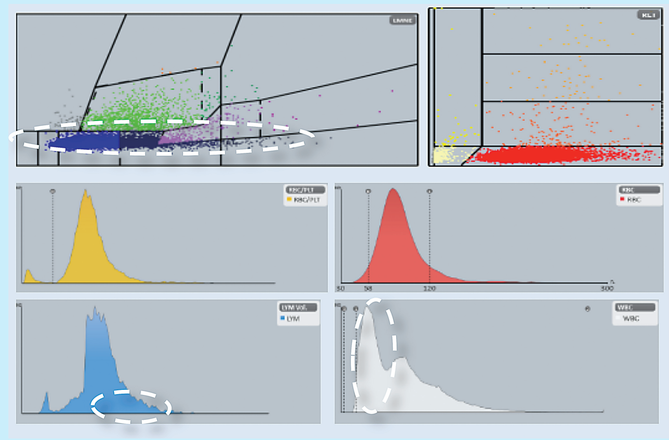
Overall agreement LYA% 92%

Interference RBC/PLT



1- On the PLT histogram, we observe no valley in the macroplatelets area. The MPV is artificially increased by the RBC interference.
2- On the RBC/PLT histogram, we observe the valley between the both population and the bad position of the threshold in impedance. The impedance value of PLT is rejected and an automatic PLTOx is more correct.
3- On the RBC histogram we observe the presence of microcytic
population. Associated with low reticulocyte response, the hypoproliferative microcytic anemia is typical of hypothyroidism.
4- On the PLTo scattergram we observe the separation between the PLT population and the RBC population in the optical axe.
The PLTOx value is correct.





1. On the DIFF scattergram we observe the presence of atypical lymphocytes and immature lymphocytic cells from the CLL.
2; On the WBC histogram, we observe the ratio of the lymphocyte population is higher than the granulocytic population.
3. On the Lymphocyte distribution histogram, we observe large cells on the right of the lymphocyte population which correspond to the large cells from the CLL. Results with smudges cells included in the lymphocyte population: NEU% 1.1, LYM% 98.7, MON% 0.2.