

# Guest Forum

## Leukocyte Differential Comparison between Pentra 120 SPS smears and Manual Slide Review



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**An evaluation was conducted to compare the leukocyte differential on smears made by the SPS to a manual slide review with 200-cell differential count (reference method).**

### Introduction

The study was designed to accomplish two aims:

- Determine the differential accuracy between the leukocyte differential on the SPS slide (test instrument) and the manual slide review (reference) over a clinical range, including normal and morphologically abnormal samples.
- Test the functionality of the SPS for laboratory work.

### Materials and Methods

The NCCLS<sup>\*1</sup> recommendations for evaluating a hematology analyzer were followed.

\*1: Global Consensus Standardization for Health Technologies

The acronym used to stand for “National Committee for Clinical Laboratory Standards,” but NCCLS is now a global organization and develops consensus documents for additional audiences beyond the clinical laboratory community. Therefore, the organization should be referred to by the acronym, “NCCLS.” (from <http://www.nccls.org/>)

### Sample

131 unselected routine normal and pathological samples were analyzed.

These samples were collected in K<sub>3</sub>EDTA<sup>\*2</sup>, maintained at room temperature and analyzed within six hours. Peripheral blood smears were prepared by the wedge method on all samples.

\*2: Tripotassium EthyleneDiamine Tetra-Acetate

## Analyzer

The Pentra 120 SPS can perform a CBC, 5-part leukocyte differential with a throughput of 120 samples per hour and a slide with the same throughput. All samples were processed in the DIFF plus slide mode.

The staining protocol used was a MGG (May Grunwald Giemsa).

All the stain was provided by HORIBA ABX.

## Protocol

An evaluation was conducted to compare smears made manually (reference method) and the ones made by the SPS:

- 131 samples studied
- for each sample, 4 smears were made as follows:
  - 1 smear made by SPS read by first evaluator
  - 1 smear made by SPS read by second evaluator
  - 1 manually-made smear read by first evaluator
  - 1 manually-made smear read by second evaluator

Total: 524 smears

One hundred (100) specimens representative of a patient population were analyzed, with 53% abnormal (erythrocyte, leucocyte, thrombocyte pathologies) and 47% normal.

## Pathology Study

**Erythrocytes:** anemia, erythrocytosis, inclusions (Jolly’s bodies, punctuate basophilia), target cells, anisocytosis, poikilocytosis, microcytes, macrocytes, hypochromasia, polychromasia, erythroblast.

**Leukocytes:** acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), Waldenstrom, myeloma, monocytosis, eosinophilia, reactive cells.

**Thrombocytes:** thrombocytosis, platelet clumps, macrothrombocytes, micromegakaryocytes.

Morphological and quantitative abnormalities taken into account in the study and present in the sample population are listed in Table 1.

Table 1 Morphological and Quantitative Abnormalities

Sample abnormalities	Criteria	Number
Erythrocyte abnormalities	· Abnormalities of form, color, size, membrane · Inclusions	27%
Platelet abnormalities	· Platelet clumps · Anisocytosis and macrothrombocytes · Micromegakaryocytes	13%
Presence of immature granulocytes	> 1% metamyelocyte	29%
Presence of other cells	· Atypical lymphocytes · Erythroblasts · Blasts	18%

200 leukocytes were counted by two independent observers each, as recommended by NCCLS document H20-A.

For each sample, one observer read one smear made by the SPS and one manually-made smear.

The comparison was carried out using the 5-part differential parameter results (neutrophils, lymphocytes, monocytes, eosinophils and basophils) as well as the other cells.

Accuracy of the leukocyte differential to the manual slide review was assessed by comparing results from the microscope examination to the Pentra 120 SPS test method.

Carryover, or slide cross contamination will be studied. One slide with chicken blood will be prepared alternately with two slides with human blood (chicken RBCs are nucleated and should be easily detected if contaminating a slide made with the human blood).

Coloration protocols are shown in Table 2.

Table 2 Coloration Protocols

Automatic Coloration	Protocol Manual Coloration Protocol*3
May Grünwald pure (ABX) 4 min Laboratory’s buffer (pH7) 2 min 30 Giemsa (ABX) at 8% 8 min Rinse buffer 1 min	May Grünwald pure 1 min Giemsa 20% (buffer pH7) 10 min Rinse

\*3: The Manual Coloration Protocol is the routine process: short protocol

## Results

### SPS Troubleshooting

During two weeks, just one technical problem was noted: one slide broke in a staining well. This was solved by autocontrol (automatic cycle).

### Correlation Graphs

The results of these studies are shown in correlation graphs (Figure 1-6) of manual mode versus automatic mode.

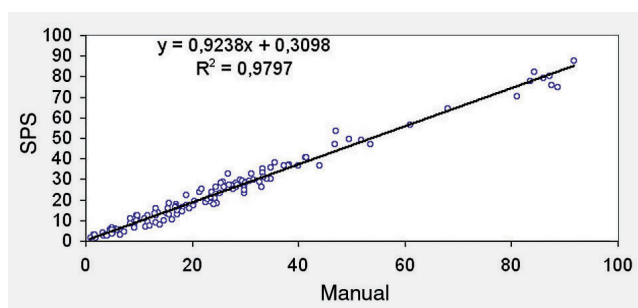


Figure 1 Correlation Lymphocytes

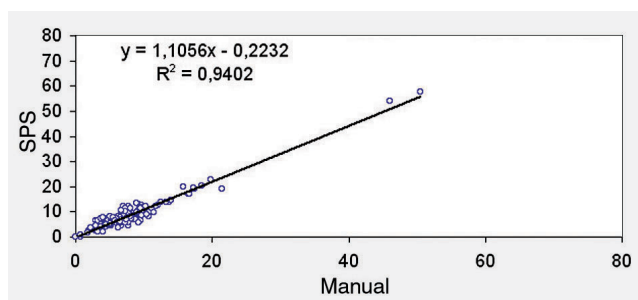


Figure 2 Correlation Monocytes

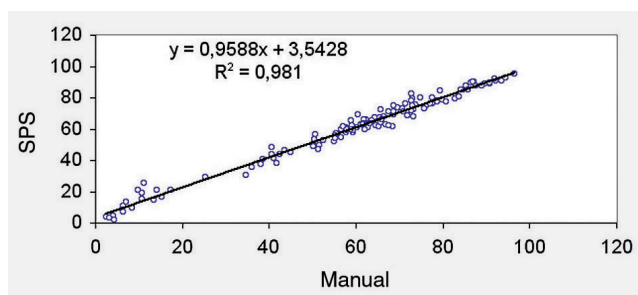


Figure 3 Correlation Neutrophils

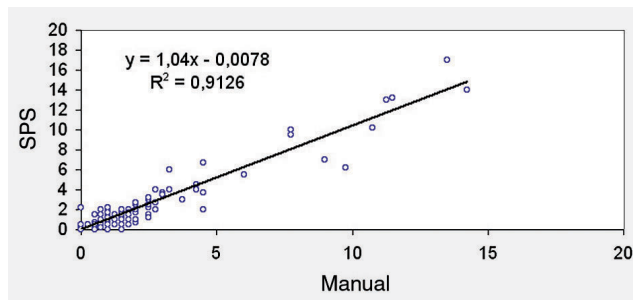


Figure 4 Correlation Eosinophils

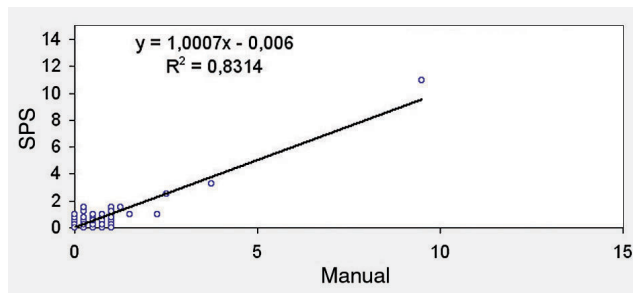


Figure 5 Correlation Basophils

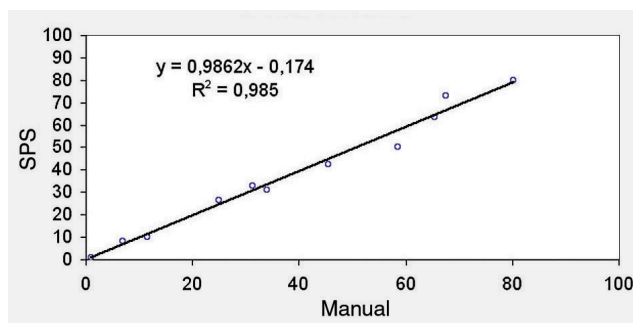


Figure 6 Correlation Blasts

Comparative results for the leukocyte differential was expressed as R<sup>2</sup> coefficients\*4 (Table 3).

Table 3 Correlation of Manual Mode versus Automatic Mode

	LY	MO	PN	PE	PB	Blasts
R <sup>2</sup>	0.98	0.94	0.98	0.91	0.83	0.98

n = 131

LY : Lymphocytes

MO : Monocytes

PN : Polynuclear Neutrophils

PE : Polynuclear Eosinophils

PB : Polynuclear Basophils

\*4:  $R^2 = 1 - \frac{\sum (Y_i - \hat{Y}_i)^2}{\sum (Y_i - \bar{Y})^2}$

$\hat{Y}_i$  : Calculated value by the regression curve

$\bar{Y}$  : Average value of  $Y_i$

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## Conclusion

### Practicability

- The Pentra 120 SPS is easy to use as it is fully automatic
- Flags are given for stain reagent levels
- Maintenance is completely automatic

### Carry Over

The results from the carryover protocol are considered acceptable if the chicken NRBCs do not contaminate any of the human blood samples. No NRBCs were observed in human samples.

### Accuracy

Correlation studies between the SPS slides and the manual slides display excellent coefficients.

The percentage of cell analysis showed excellent results except for CLL (increase of neutrophils in SPS smears because some lymphocytes were broken).

### Quality of the Slides

The reading area of the SPS smear is larger than that of the manual smear.

No cell concentration was observed on the smear edge.

The staining quality with MGG is very good.

For laboratory work, the SPS gave excellent qualitative and quantitative results compared to the manual mode. Its throughput allows for quick, regular and reliable work.