



## TECHNOLOGY AND CASE STUDIES »

### DxH 500\* Hematology Series



» Move healthcare forward.

\*Not available for sale in the U.S.



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# DxH 500 SYSTEM TECHNOLOGY

The DxH 500 Hematology System technology incorporates the Coulter Principle and direct optical measurements to provide an effective and robust technology for cellular analysis.

## » The Coulter Principle<sup>1</sup>

The Coulter Principle is an electronic method for counting and sizing particles. Although the Coulter Principle can be used to count and size just about any type of particle, the specific application of this principle in hematology is to count and size White Blood Cells, Red Blood Cells, and Platelets.

## » Electronic counting and sizing basics

The Coulter Principle (impedance) is used to count and size cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) is suspended in a conductive liquid and passes through a small aperture. As each cell goes through the aperture, it acts as an insulator and it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture (Figure 1). This causes a measurable electronic pulse. A regulated vacuum is used to pull the diluted cell suspension through the aperture for counting. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

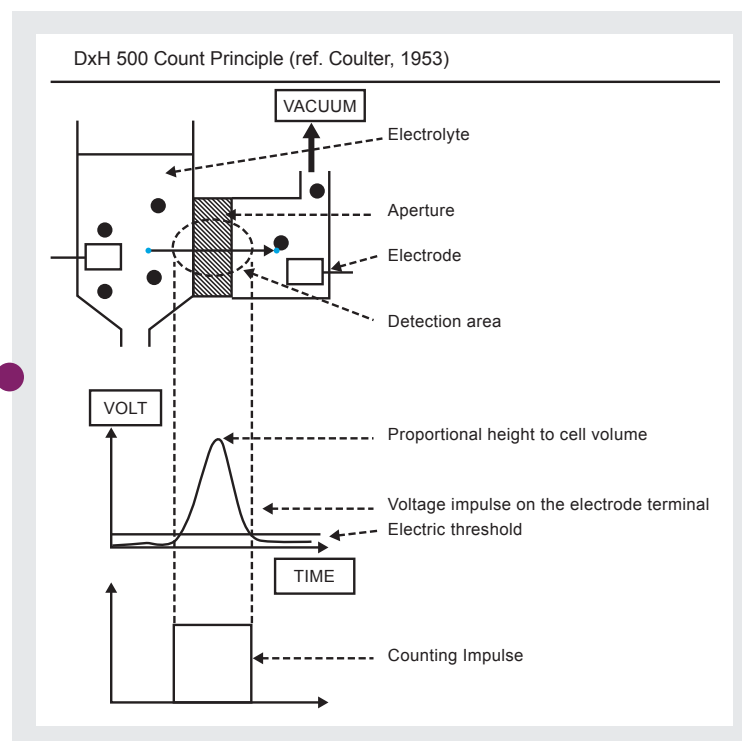


Figure 1. DxH 500 Coulter Principle

### › DxH 500 System Overview

The DxH 500 whole blood sample preparation and count process are detailed below.

- 12  $\mu$ L of whole blood is sample aspirated.
- Sample probe retracts, external probe surface is rinsed with DxH 500 Diluent.
- Sample probe moves above the White Blood Cell (WBC) bath and the sample probe's external surface is rinsed again. The WBC bath is drained.
- 1.0 mL of DxH 500 Diluent is dispensed into the clean and empty WBC bath.
- An additional 0.5 mL of DxH diluent is dispensed through the sample probe pushing the 12  $\mu$ L of sample into the bath and creating the initial WBC dilution (1:125 Blood:Diluent).
- The WBC dilution is mixed using air bubbles.
- Probe enters the WBC dilution and aspirates 25  $\mu$ L of the initial WBC dilution to be used for the RBC dilution.
- Probe retracts and the external surface is rinsed.
- As the sample probe moves above the Red Blood Cell (RBC) bath, 0.66 mL of DxH 500 Lyse is dispensed into the WBC bath to lyse RBC and create the final WBC dilution (1:180 Blood:Diluent/Lyse)
- The Lysed WBC dilution is air mixed in preparation for analysis.
- The WBC dilution is used for the counting and differentiation of the WBCs and measurement of hemoglobin.
- 1.5 mL of DxH 500 Diluent is dispensed into the clean and empty RBC bath.
- 0.5 mL of DxH 500 Diluent is dispensed through the sample probe, pushing the 25  $\mu$ L of the initial WBC dilution into the RBC bath, creating the final RBC dilution. (1:10000 Blood:Diluent)
- The RBC dilution is mixed and prepared for the counting and sizing of the RBCs and PLTs.
- The system performs two count measurements:
  - 3 sec count period = Resistive (counts) (RBC/PLT/WBC)
  - 7 sec count period = Resistive (counts) (RBC/PLT/WBC) & Resistive and Optical DIFF
- Vacuum is generated using the dual syringe system.
- The syringe system is pre-charged for each counting cycle.
- Before all counting cycles, the vacuum level is checked.
  - The generated vacuum is compensated for high altitudes.
- The apertures are cleaned between counting phases.



### › Counting/Sizing

The RBC and WBC counts are determined using the Coulter Principle to accurately count and size cells. The WBC differential is determined using a combination of the impedance WBC data and the direct optical measurement data obtained using a blue LED focused through the WBC aperture.

### › Coincidence Correction

More than one cell may occasionally pass through the aperture sensing zone at the same time. When cells coincide, only one combined pulse is counted. Because the frequency of coincidence is proportional to the actual count, the system automatically corrects results for coincidence.

### › Voting

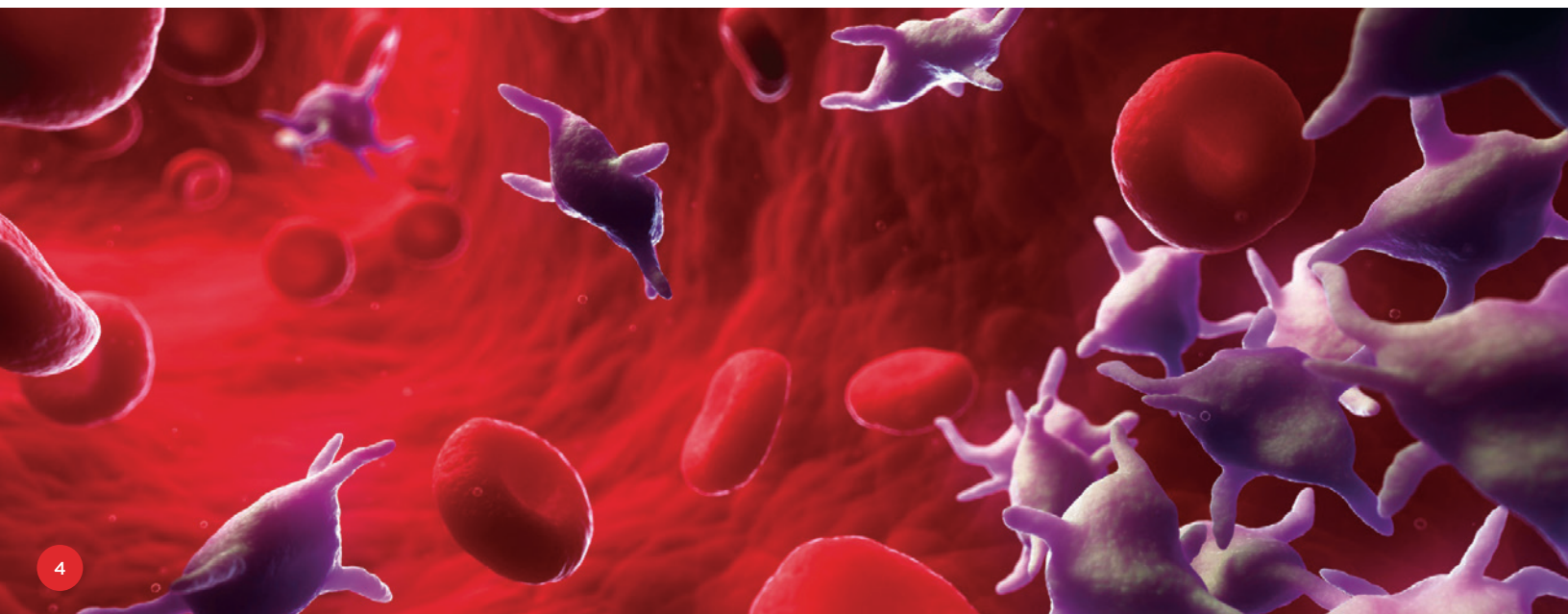
To prevent data errors due to statistical outliers or obstructions that may block an aperture, the system votes on data for WBC, RBC, and PLT. The system verifies that the data produced is within an established statistical range and is used to generate parameter results.

### › Scaling

Scaling adjusts for calibration and reportable format.

### › Hemoglobinometry

Human whole-blood hemoglobin is converted into stable oxyhemoglobin by the DxH 500 Lyse reagent. The resulting complex is then measured by spectrophotometry using an LED  $\lambda=545\text{nm}$ , but with a  $\delta\lambda=20\text{nm}$  band width. The absorbance is integrated on this 40nm band around  $\lambda=545\text{nm}$ . The resulting hemoglobin measured by absorbance includes oxyhemoglobin.



## » Parameter Derivation

### > WBC count

The WBC count is measured directly by counting all particles in the WBC dilution. The DxH 500 lytic reagent removes red blood cells. Platelets are removed below a predefined threshold. The final WBC count is provided after performing the coincidence correction, voting, and multiplication by a calibration factor.

### > WBC Differential

The WBC differential (5-part) is determined using the simultaneous measurements of impedance (volume) and direct optical (Axial Light Loss) within the WBC aperture. The DxH 500 WBC differential technology uses an aperture of proprietary design. The aperture optical assembly is placed perpendicular to the aperture. The LED in the optical assembly projects a blue light through the aperture wall and onto a sensor that detects axial light loss. As cells pass through the aperture, the optical path is interrupted. The amount of light falling on a sensor can be measured and varies depending on cell structure (see Figure 2).

Cells passing through the center of the aperture generate Gaussian pulses by impedance. Cells that are not centered will produce non-Gaussian pulses (see Figure 3). Gaussian pulses (T1, T2, and T3 in Figure 3) that pass through the center are positioned properly within the aperture for the optical measurement. Gaussian pulses and axial light loss are further analyzed by the DxH 500 algorithm to generate the WBC differential, flagging, and messaging. A two-dimensional scatterplot is created by placing volume on the Y-axis and axial light loss on the X-axis. The scatterplot shows differentiation of white cells into five major classifications: Lymphocytes, Monocytes, Neutrophils, Eosinophils, and Basophils. Non-Gaussian pulses (T4 in Figure 3) are discarded.

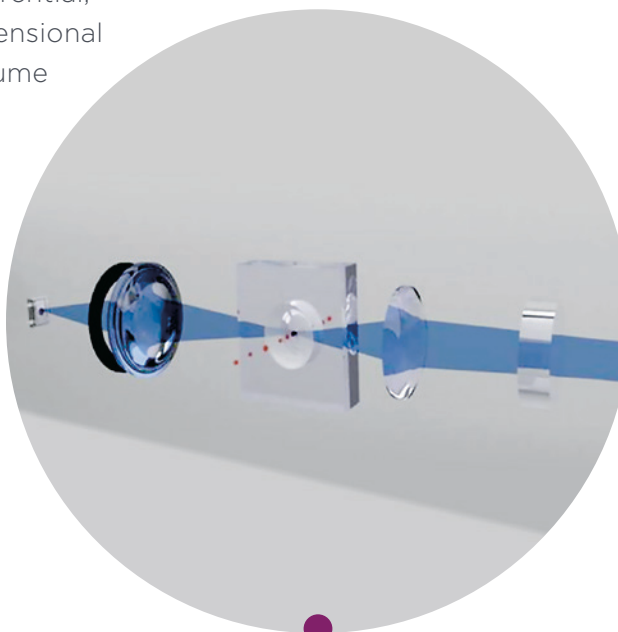


Figure 2. DxH 500 WBC Differential Impedance / Optical measurement

## DXH 500 DIFFERENTIAL MEASUREMENT

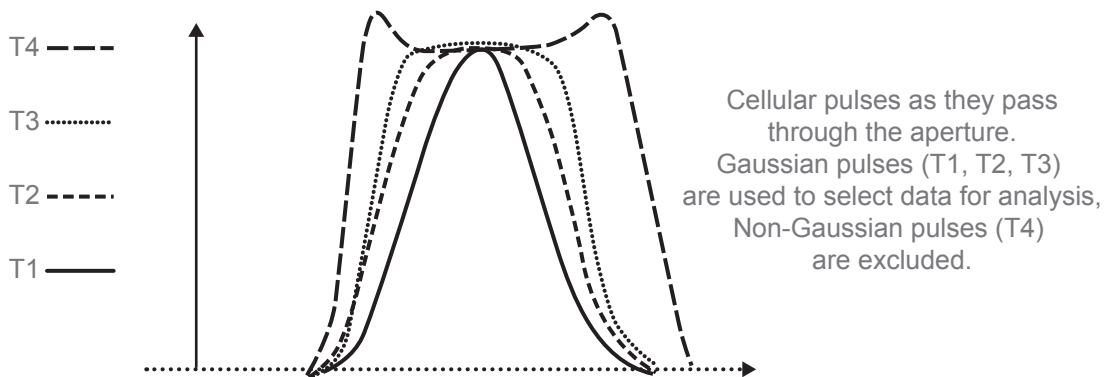
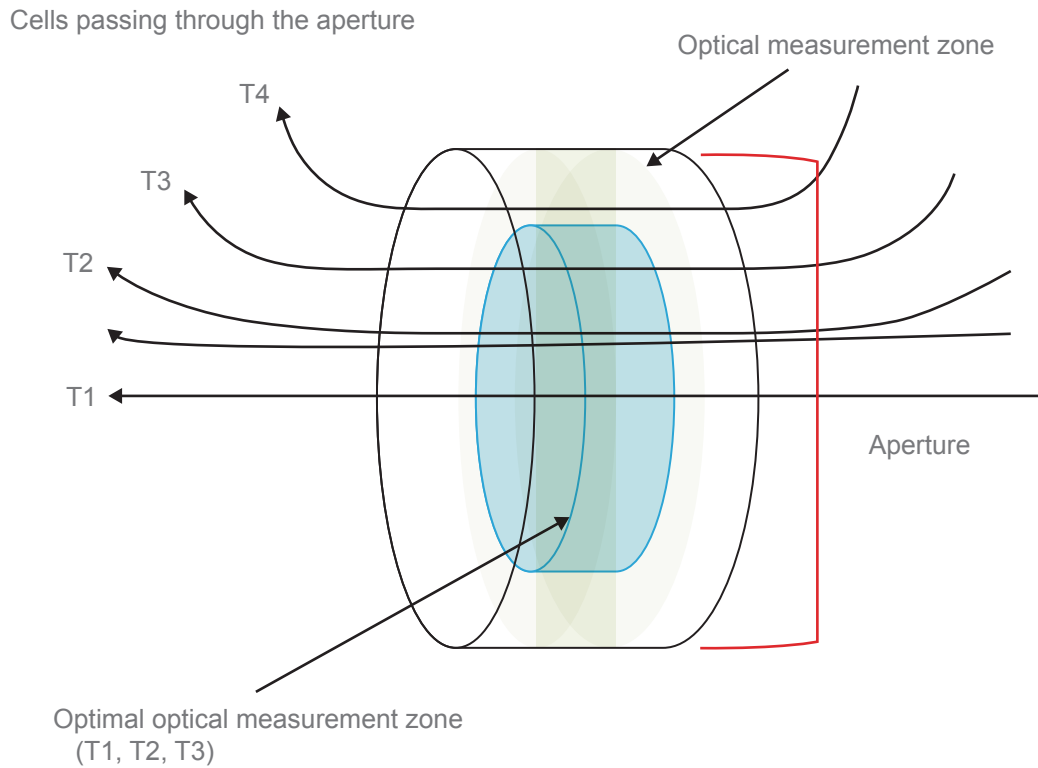


Figure 3. DxH 500 WBC Differential Impedance/Optical Measurement



### › Hemoglobin concentration

HGB concentration is a directly measured parameter. The released hemoglobin in the WBC bath is converted into stable Oxyhemoglobin (Carboxyhemoglobin, if present). An LED is used to measure the solution by spectrophotometry at  $\lambda=545\text{nm}$ . The absorbance of the sample is compared to a blank reading, a calibration factor is applied, and the hemoglobin concentration result is reported.

### › RBC Count

The RBC count is a directly measured parameter. The RBC dilution contains red blood cells, white blood cells, and platelets. Thresholds are used to separate the smaller platelet pulses from the red and white blood cell pulses.

The white blood cells present in the dilution are included in the red blood cell count, but their interference is insignificant because there are only a few thousand white blood cells in comparison to millions of red blood cells. After coincidence correction and voting, the analyzer multiplies the RBC count by a calibration factor and reports the result.

### › RBC Histogram

The RBCs are categorized according to size by a pulse-height analyzer. Particles are sorted into 256 (volume) channels to develop a histogram. The display range is approximately 25 to 360 fL and the system monitors the area at the lower end of the histogram for interferences. In the presence of interferences, the algorithm will determine the degree of interference and correct the results. The system will flag the results in cases of severe interference.

### › Mean Corpuscular Volume

The MCV is derived from the RBC histogram. It is the average size of all cells in the RBC histogram. After coincidence correction and voting, the MCV is multiplied by a calibration factor and the result is reported.

› Hematocrit

The Hct is a calculated parameter and is the relative volume of packed erythrocytes to whole blood, expressed as a percentage. The formula is:

$$\text{HCT (\%)} = \frac{\text{RBC} \times \text{MCV}}{10}$$

› Mean Corpuscular Hemoglobin

The MCH is calculated and indicates the average weight of hemoglobin in the red blood cell. The formula is:

$$\text{MCH (pg)} = \frac{\text{HGB} \times 10}{\text{RBC}}$$

› Mean Corpuscular Hemoglobin Concentration

The MCHC is an expression of the average concentration of hemoglobin in the red blood cells. It relates the average amount (mass) of hemoglobin in the red blood cells to the average volume of the red blood cells. It is computed using the formula:

$$\text{MCHC (g/dL)} = \frac{\text{HGB} \times 100}{\text{HCT}}$$

› Red Cell Distribution Width

The RDW is a measure of the variability in the size of the red cells derived from the RBC histogram. The analyzer uses the cells from the distribution curve to calculate the coefficient of variation of the size of the cells and is expressed as a percentage:

$$\text{RDW} = \frac{\text{Standard Deviation} \times 100}{\text{Mean Size}}$$

› Red Cell Distribution Width—SD

The RDW-SD size is the distribution spread of the erythrocyte population derived from the RBC histogram and expressed as a standard deviation in fL.

### › Platelet Count

The platelet count is derived from an internal continuous PLT/RBC histogram. Particles between 0 and 70 fL are counted and sized as they pass through the RBC aperture. The raw data is evaluated using proprietary DxH platelet algorithms to identify the platelet population. The system also performs feature analysis for the identification of patterns of interference at the low and high ends of the PLT histogram. The algorithm uses both the PLT raw data and the fitted histograms for this process to determine PLT interference patterns, correcting or flagging results, depending on the severity of the interference. The platelet histogram's evaluation improves accuracy by excluding interferences from debris, micro bubbles, red cell fragments or exceptionally small red blood cells.

### › Mean Platelet Volume

MPV represents the average size of the platelets derived from the platelet histogram. The instrument then multiplies by a calibration factor.

### › Histograms and Diff Plots

The histograms show relative cell frequency (Y-axis) versus size (X-axis). They provide information about red cell and platelet frequency. Histograms can provide a means of comparing the sizes of a patient's cells with normal populations.

## » IMPORTANT

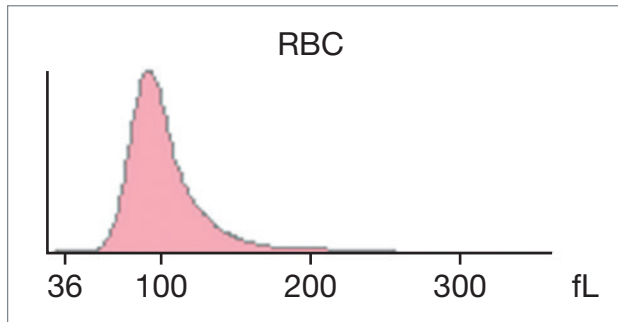
Histograms show only the relative, not actual, number of cells in each size range. Do not estimate the number of cells from the histograms.

Selecting a histogram on the user interface displays a larger view of the histogram. Each histogram is grey with a white background. Each cell population is shaded as follows:

- RBC - light red
- PLT - light green



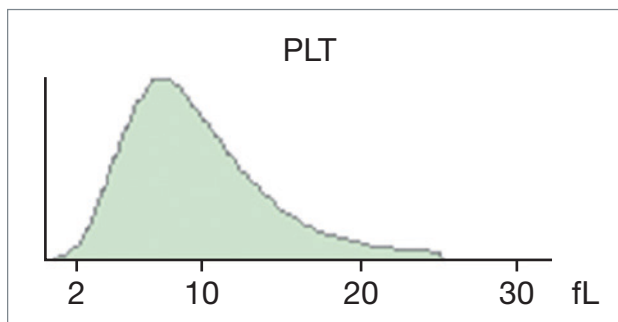
# HISTOGRAMS AND DIFF PLOTS



Normal RBC Histogram

## Typical RBC Histogram

- The main population is a bell-shaped, symmetrical curve.
  - The smallest population to the right of the main population represents the RBC doublets, triplets and WBCs.
- › Normal characteristics - RBC Histogram
- There is a clear baseline below 50 fL and the curve is between 50 and 200 fL
  - The RBC curve has a slight skew to the right
  - There is a clear unimodal mode
  - The width of the RBC curve is normal; the RDW is likely normal



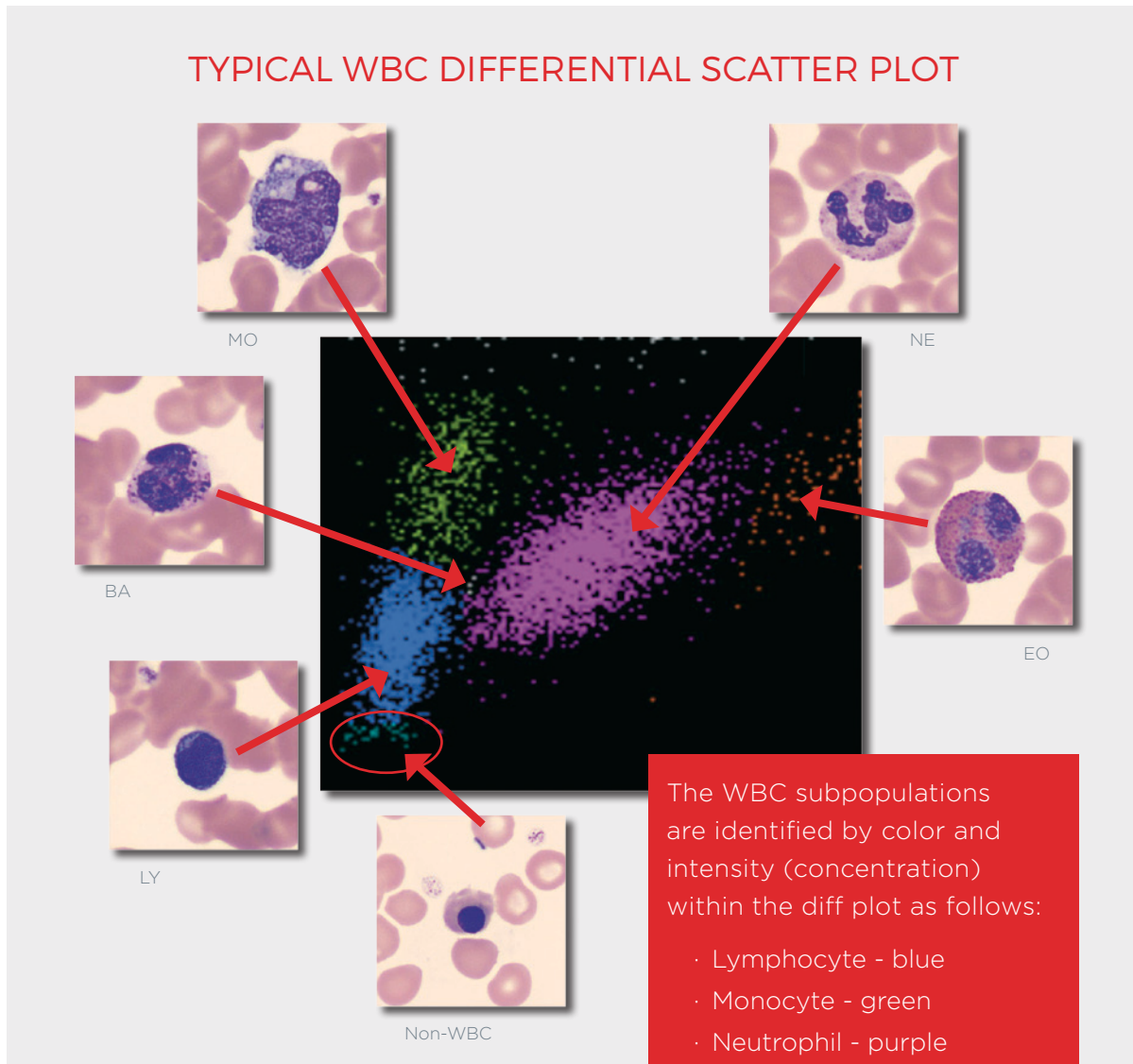
Normal PLT Histogram

## Typical Platelet Histogram

- The PLT histogram is evaluated for patterns of interference at the low and high ends.
  - The internal PLT algorithm uses raw data and fitted histograms to determine the patterns.
- › Normal characteristics - PLT Histogram
- Log normal
  - Low baseline at 2 fL, normally extending to approximately 25 fL
  - Positive log-normal distribution
  - Mode between 3 and 15 fL

## » Differential Scatter Plot (Diff Scatter Plot) Development

The digital information obtained from the WBC differential analysis is processed through the WBC differential algorithm. This information is represented on a 2D scatter plot, with cell volume plotted on the Y-axis and Axial Light Loss (ALL) plotted on the X-axis.



## » DxH 500 SYSTEM FLAGGING OVERVIEW

Flags, codes, and messages are evaluated when the sample is analyzed. Review the results and pay close attention to any flags, codes, or messages that are intended to alert you to issues with results or with the instrument. Look for data patterns when examining flags, codes, and messages. Determine if individual or sets of results (for example, WBC and differential results) exhibit flags, codes, and messages. Some flagging occurs as a result of the flagging or editing of other parameters. In all cases, follow your laboratory's policy for reviewing results.

### » Flags

Flags appear to the right of the parameter result. Flagging occurs as a result of the flagging limits, system messages, or editing of parameters. When flagging limits change, flags are not reevaluated for results that are already in the database.

The flags shown in the following table are listed in order of priority, from highest to lowest. The columns indicate the three positions where flags appear. It is possible to have flags in all or each of the three positions.



Flag and Position			Description
1	2	3	
E			Manual edit of a primary parameter
e			Automatic edit of a calculated parameter
+			Result is above the analytical measuring range high limit
-			Result is below the analytical measuring range low limit
	R		Review results
	*		Hemoglobin and Hematocrit (H&H) check failure (Hct - 3) < (Hgb*3) < (Hct + 3)
		H	<ul style="list-style-type: none"> <li>· Patient results above the action limit</li> <li>· Control results above the expected range</li> </ul>
		L	<ul style="list-style-type: none"> <li>· Patient results below the action limit</li> <li>· Control results below the expected range</li> </ul>
		h	Patient results above the reference interval, but less than the action limit (H)
		l	Patient results below the reference interval, but less than the action limit (L)

**Table 1.** DxH 500 System Flags and Position Description

## » Codes

Codes are non-numeric characters that appear in place of values when the system cannot generate results.

## » IMPORTANT

Beckman Coulter recommends that you review all flags and codes according to your laboratory's protocol.

The codes in the following table are listed from highest to lowest priority.

Code	Description
-----	Total vote out (dashes). Inconsistent data between count periods.
.....	Incomplete computation (dots). Data cannot be derived.
+++++	Above operating range (plus signs).
?????	Result is outside the range of values that can be formatted for display (question marks).

**Table 2.** DxH 500 System Codes



## » System Messages

All messages are accompanied by R (Review) flags or other flags. A system message indicates an event occurrence that may affect the operation of the system, require operator notification, or entry into an Event Log.

Refer to Table 3 and Figures 4, 5, and 6 for all System Messages.

## » **IMPORTANT**

Beckman Coulter recommends that you review and handle all messages according to your laboratory's protocol.



System Message	Description
BA Interference	Multiple populations are overlapped. Cannot calculate BA%. The R flag appears next to Diff% and # results. A non-numeric (.....) appears for BA% and BA#.
Cellular Interference	Poor separation between WBC population and interference in the lower lymphocyte area. The R flag appears next to WBC and/or Plt, and DIFF%/# results. The number of cells is below the WBC count threshold.
Debris	Too many events in the debris area.
Dimorphic RBC	Evidence of the presence of at least two populations of red cells. The RBC Histogram flags affect the RDW results. This flag is inhibited when both WBC and RBC are less than the measuring range, or when the RBC result is non-numeric (+++++ or .....). The R flag appears next to RDW and RDW-SD.
H & H Check Failed	The ratio of HGB to HCT is not in the expected range $(Hct - 3) < (Hgb \times 3) < (Hct + 3)$ . The * flag appears next to HGB, HCT, and the computed related results.
Hgb Blank Error	HGB blank reading is outside the internal threshold limits. The HGB and computed result is non-numeric (.....).
Large Cells	High number of events in the large cell area. The R flag appears next to Diff% and # results.
Low Diff Events	Not enough good white events during Diff analysis. The scatter plot total numbers of cells is less than 500. The R flag appears next to Diff% and # results.
LY/MO Overlap	Lymphocyte and Monocyte populations are overlapped. The R flag appears next to Diff% and # results.
MO/NE Overlap	Monocyte and Neutrophil populations are overlapped. The R flag appears next to Diff% and # results.
NE/LY Overlap	Neutrophil and Lymphocyte populations are overlapped. The R flag appears next to Diff% and # results.
NE/EO Overlap	Neutrophil and Eosinophil populations are overlapped. The R flag appears next to Diff% and # results.
PLT1 - Debris	Interference with smaller platelets. Interference at the left side of the PLT histogram between channel 0 and the CP1 threshold.
PLT2 - Debris	Interference with larger platelets. Interference at the right side of the PLT histogram between the CP2 and P thresholds.
PLT3 PLT/RBC Overlap	PLT and RBC populations are overlapped between the CP3 and CP3-2 thresholds.
WBC/DIFF Carryover	The estimated WBC carryover, based on the WBC value from the preceding sample and the expected WBC carryover percent, may significantly affect the WBC results for the current specimen. The R flag appears next to WBC, Diff% and # results.
PLT Carryover	The estimated PLT carryover, based on the PLT value from the preceding sample and the expected PLT carryover percent, may significantly affect the PLT results for the current specimen. The R flag appears next to PLT and related results.
RBC Aggregates	MCH, RDW, and RDW-SD all exceed threshold limits. The R flag appears next to RBC, MCH, RDW, and RDW-SD.

Table 3. System Messages

## » System Messages – RBC and PLT Histograms

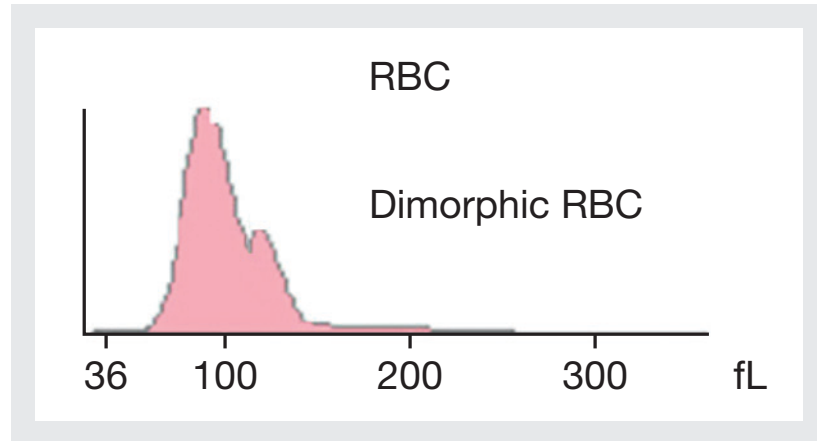


Figure 4. RBC Histogram Messages

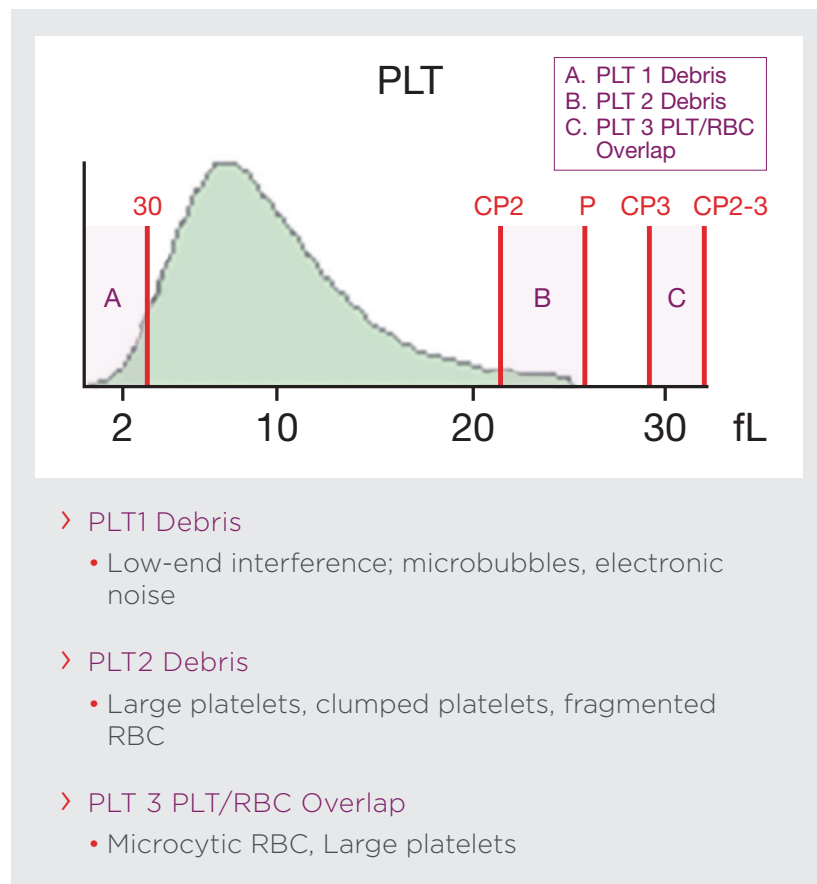


Figure 5. PLT Histogram Messages

## System Messages - WBC Differential Scatterplot

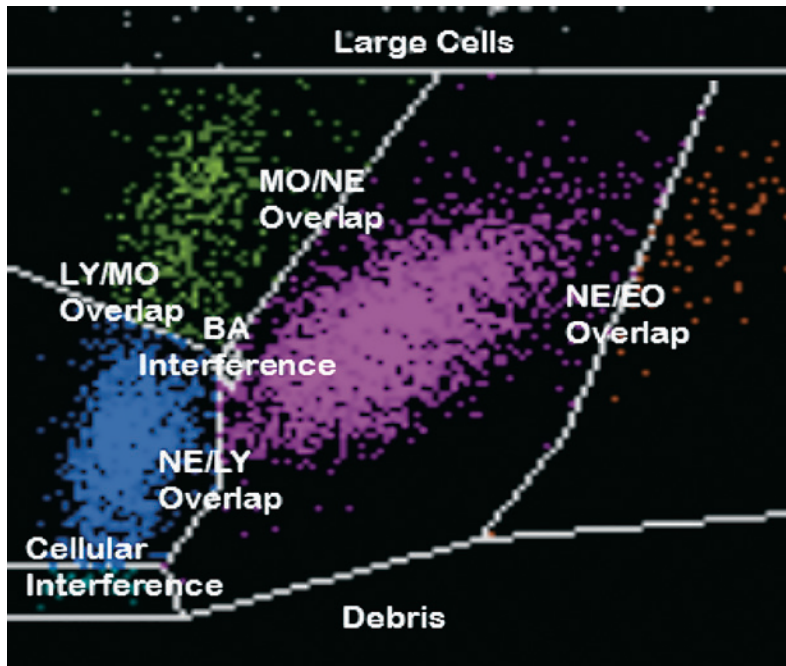


Figure 6. Scatter Plot Messages

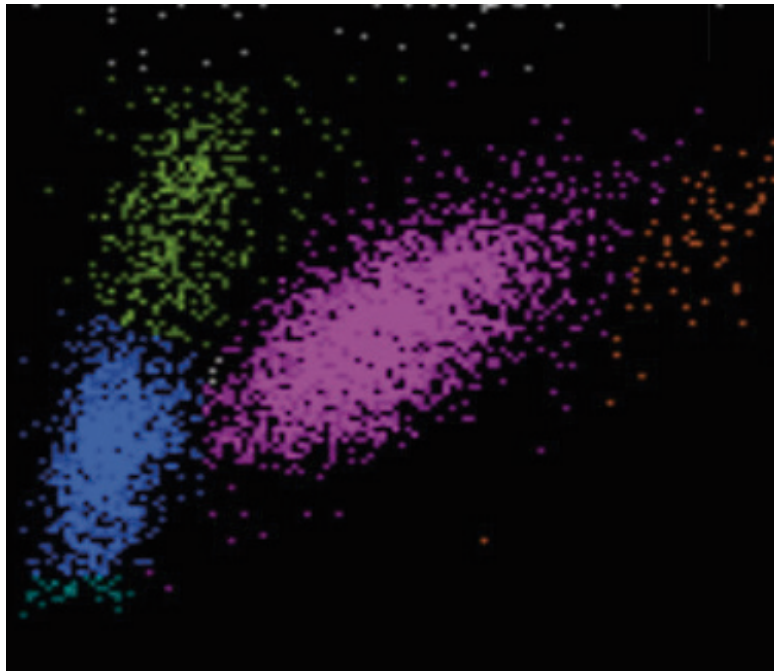
- > Debris
  - PLT aggregates, NRBC, Lyse-resistant RBC
- > Cellular Interference
  - PLT aggregates, NRBC, Small Lymphocytes, Lyse-resistant RBC
- > NE/LY Overlap
  - Small Neutrophils (hypo-granular), Abnormal Lymphocytes
- > BA Interference
  - Abnormal / Small Monocytes, Neutrophils
- > LY/MO Overlap
  - Atypical Lymphocytes, Blasts
- > MO/NE Overlap
  - Bands: Immature Neutrophils, Immature Granulocytes
- > NE/EO Overlap
  - Atypical Neutrophils, Immature Eosinophils, Agranular Eosinophils
- > Large Cells
  - Blasts, Immature Neutrophils, Immature Monocytes



# CASE 1 – NORMAL

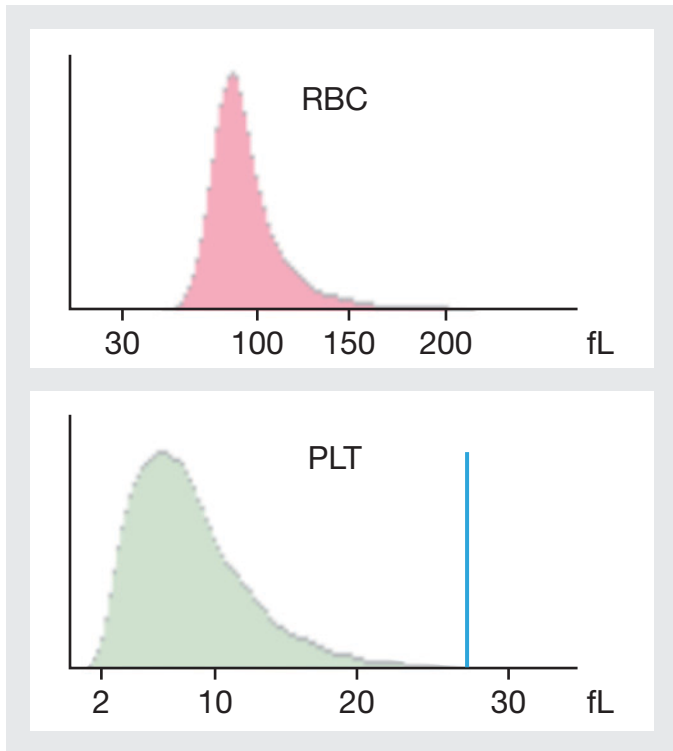
- > No instrument codes, flags and messages observed.
- > Scatter plot and histogram populations appear normal.

WBC Differential Results			
WBC	4.9		$\times 10^3/\mu\text{L}$
LY	26.5		%
MO	8.6		%
NE	63.0		%
EO	1.8		%
BA	0.1		%
LY#	1.3		$\times 10^3/\mu\text{L}$
MO#	0.4		$\times 10^3/\mu\text{L}$
NE#	3.1		$\times 10^3/\mu\text{L}$
EO#	0.1		$\times 10^3/\mu\text{L}$
BA#	0.0		$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot

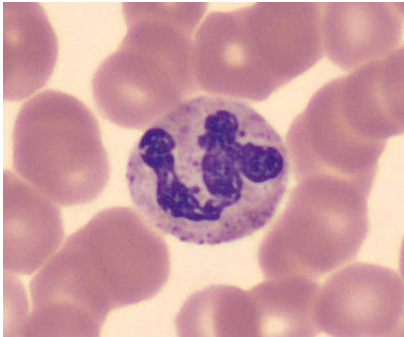
RBC Results			
RBC	5.07		$\times 10^6/\mu\text{L}$
HGB	16.2		g/dL
HCT	47.4		%
MCV	93.4		fL
MCH	32.0		pg
MCHC	34.2		g/dL
RDW	12.7		%
RDW-SD	40.4		fL



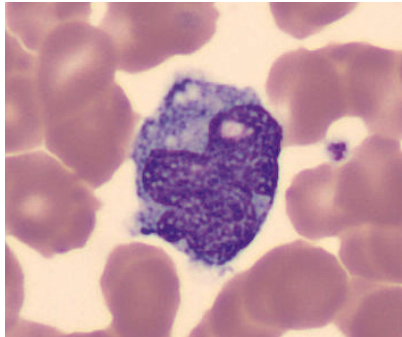
PLT Results			
PLT	228.7		$\times 10^3/\mu\text{L}$
MPV	8.4		fL

The scatter plot and histogram populations appear normal.

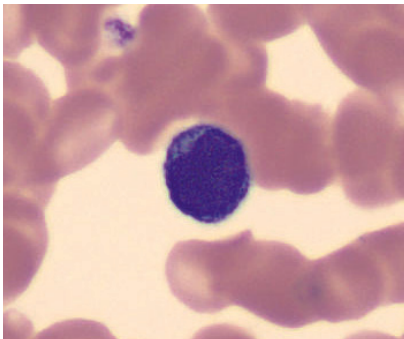
## » Blood Smear (CellaVision DM96)



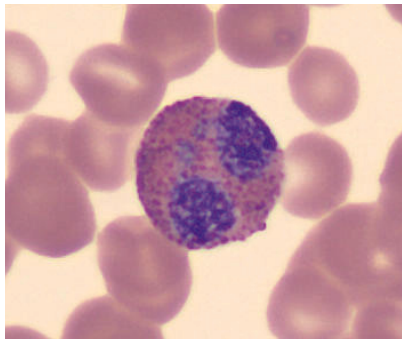
NE



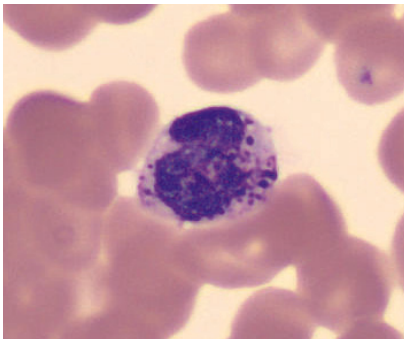
MO



LY



EO



BA

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	
Segmented Neutrophils	61
Eosinophils	3
Basophils	2
Prolymphocytes	
Lymphocytes	29
Atypical Lymphocytes	
Promonocytes	
Monocytes	5
Plasma Cells	
NRBCs	

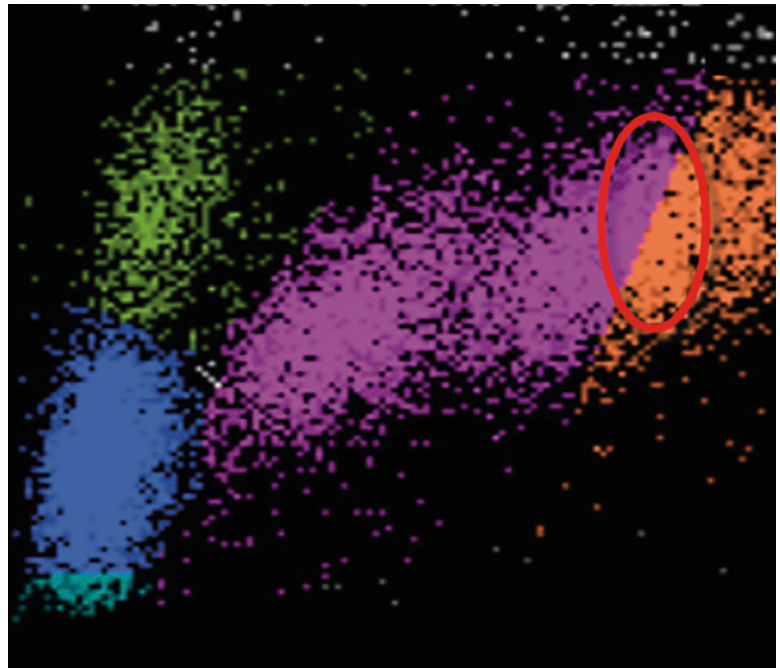
## » Summary Results

- All values are within reference ranges.
- This case illustrates a sample with normal results and no observed abnormalities.

## CASE 2 – EOSINOPHILIA

- › Instrument Messages: Abnormal Diff, NE/EO Overlap Messages; Differential results “R” flagged; Hemoglobin results “L”

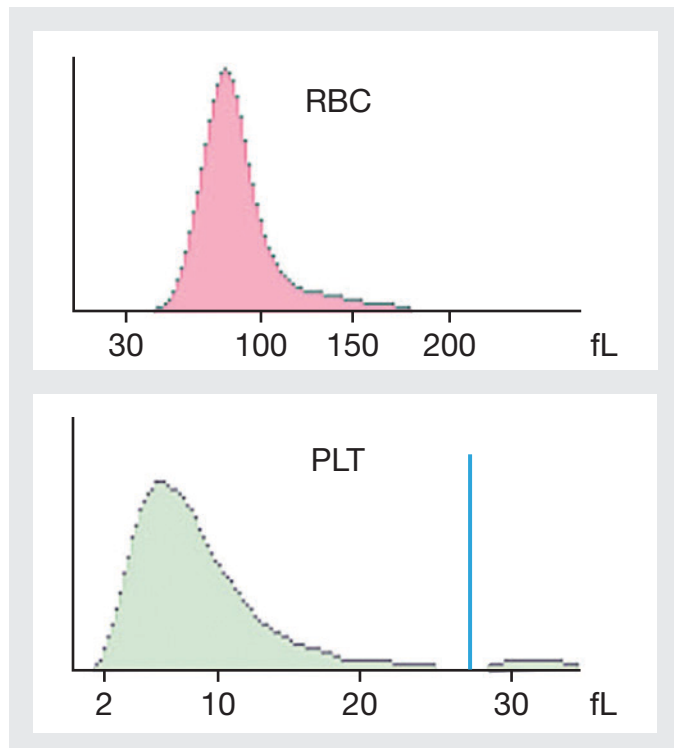
WBC Differential Results			
WBC	11.1		$\times 10^3/\mu\text{L}$
LY	32.6	R	%
MO	6.6	R	%
NE	42.5	RL	%
EO	18.2	RH	%
BA	0.1	R	%
LY#	3.6	R	$\times 10^3/\mu\text{L}$
MO#	0.7	R	$\times 10^3/\mu\text{L}$
NE#	4.7	R	$\times 10^3/\mu\text{L}$
EO#	2.0	RH	$\times 10^3/\mu\text{L}$
BA#	0.0	R	$\times 10^3/\mu\text{L}$



DxH 500 Scatter plot - NE/EO Overlap

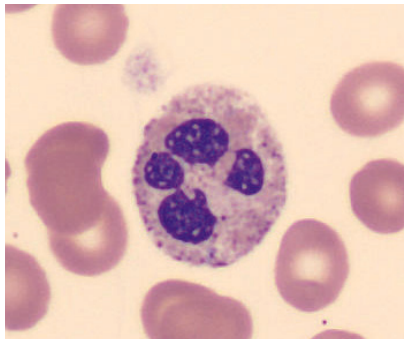
RBC Results			
RBC	2.91	I	$\times 10^6/\mu\text{L}$
HGB	8.2	L	g/dL
HCT	25.3	I	%
MCV	87.0		fL
MCH	28.2		pg
MCHC	32.4		g/dL
RDW	14.2		%
RDW-SD	39.9		fL

PLT Results			
PLT	168.5		$\times 10^3/\mu\text{L}$
MPV	8.7		fL

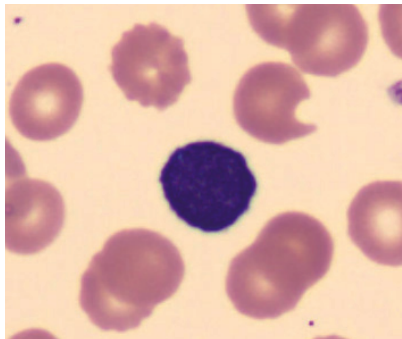


The differential scatter plot displays population of cells extending from the Neutrophil region into the Eosinophil region. Differential results are flagged for review. RBC and PLT histograms appear normal.

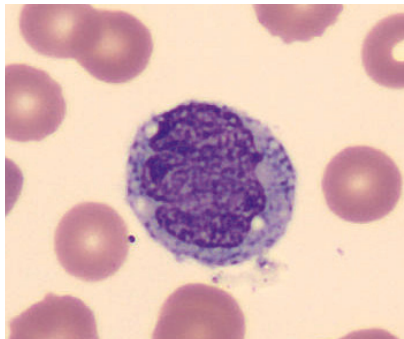
## » Blood Smear (CellaVision DM96)



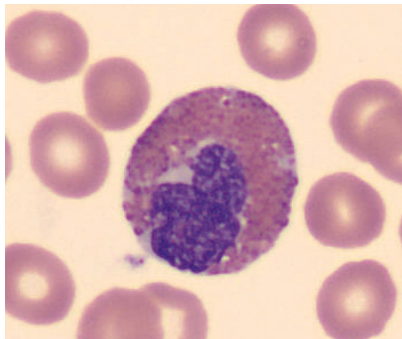
NE



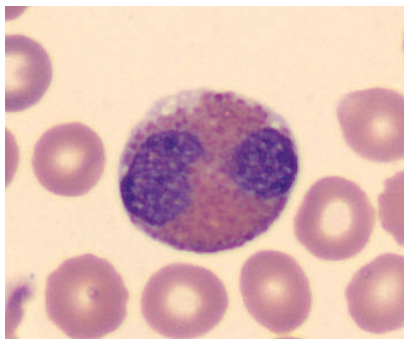
LY



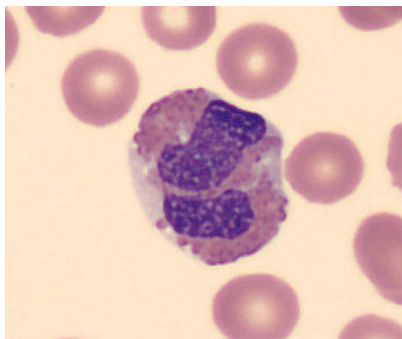
MO



EO



EO



EO

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	1
Segmented Neutrophils	24
Eosinophils	37
Basophils	
Prolymphocytes	
Lymphocytes	36
Atypical Lymphocytes	
Promonocytes	
Monocytes	2
Plasma Cells	
NRBCs	

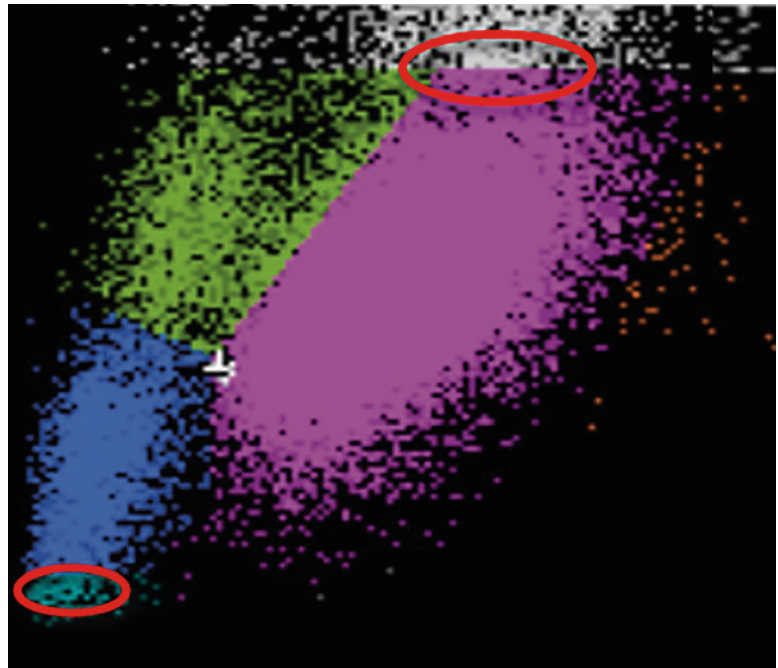
## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- Eosinophils are elevated, with some appearing slightly hypo-granular and with vacuoles.
- Morphology: RBCs appear normal in size, slightly hypochromic.
- This case illustrates a sample with eosinophilia and hypochromia.

# CASE 3 – LEUKOCYTOSIS, IMMATURE NEUTROPHIL

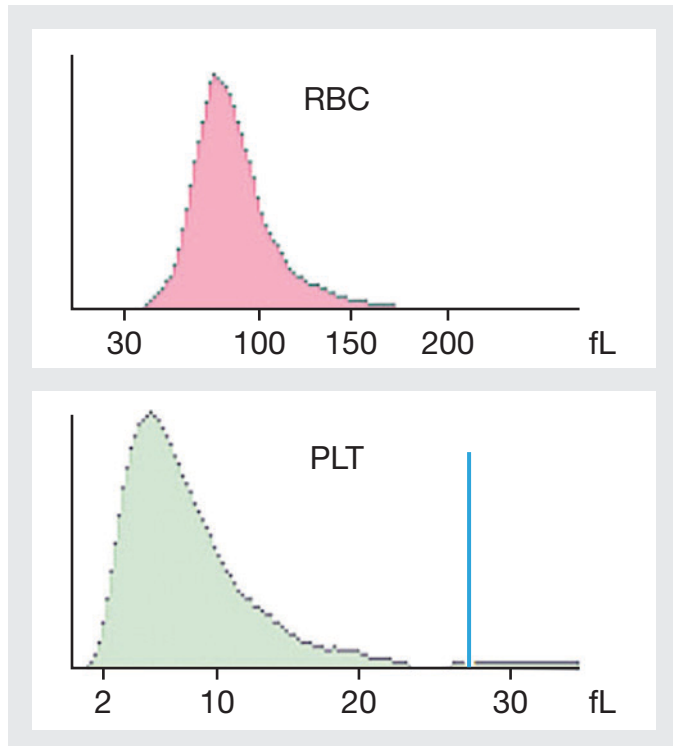
- Instrument Messages: Abnormal Diff, Cellular Interference, Large Cells; WBC and differential flagged “R”; RBC, Hgb, Hct flagged “L”; PLT flagged “R”

WBC Differential Results			
WBC	44.8	RH	$\times 10^3/\mu\text{L}$
LY	8.0	RL	%
MO	7.9	R	%
NE	83.8	Rh	%
EO	0.2	R	%
BA	0.1	R	%
LY#	3.6	R	$\times 10^3/\mu\text{L}$
MO#	3.5	RH	$\times 10^3/\mu\text{L}$
NE#	37.5	RH	$\times 10^3/\mu\text{L}$
EO#	0.1	R	$\times 10^3/\mu\text{L}$
BA#	0.0	R	$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot with Cellular Interference, Large Cells

RBC Results			
RBC	2.12	L	$\times 10^6/\mu\text{L}$
HGB	6.7	L	g/dL
HCT	20.1	L	%
MCV	94.7		fL
MCH	31.6		pg
MCHC	33.3		g/dL
RDW	17.0	h	%
RDW-SD	55.2		fL

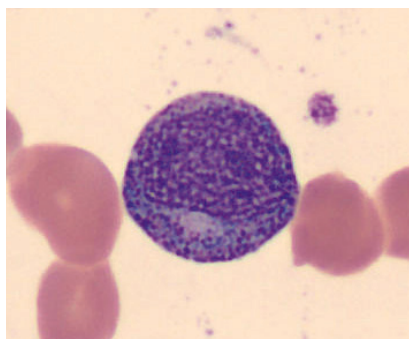


PLT Results			
PLT	355.8	R	$\times 10^3/\mu\text{L}$
MPV	8.3		fL

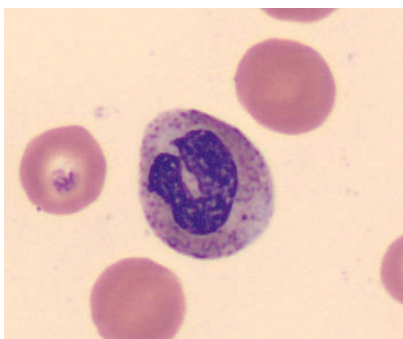
The differential scatter plot displays population of cells extending from the Neutrophil region into the Large Cell region and also from the Lymphocyte region into Cellular Interference region. Numerous results are flagged for review.



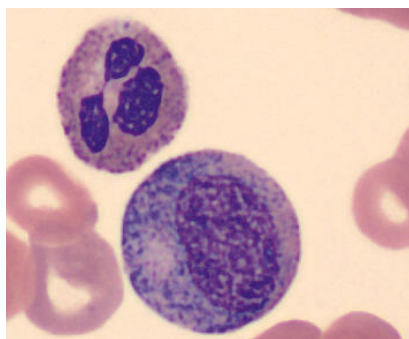
## » Blood Smear (CellaVision DM96)



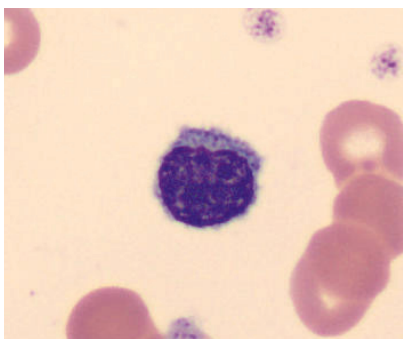
PROMYELO



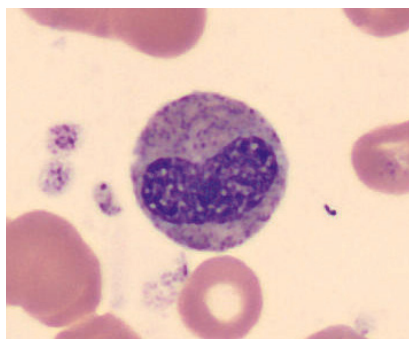
BAND



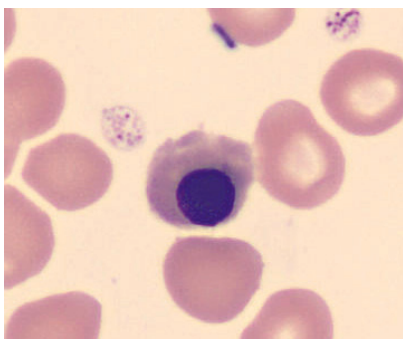
MYELO, NE



LY



META



NRBC

### Manual Differential

Blasts	1
Promyelocytes	1
Myelocytes	6
Metamyelocytes	4
Bands	29
Segmented Neutrophils	49
Eosinophils	
Basophils	
Prolymphocytes	
Lymphocytes	6
Atypical Lymphocytes	
Promonocytes	
Monocytes	4
Plasma Cells	
NRBCs	3

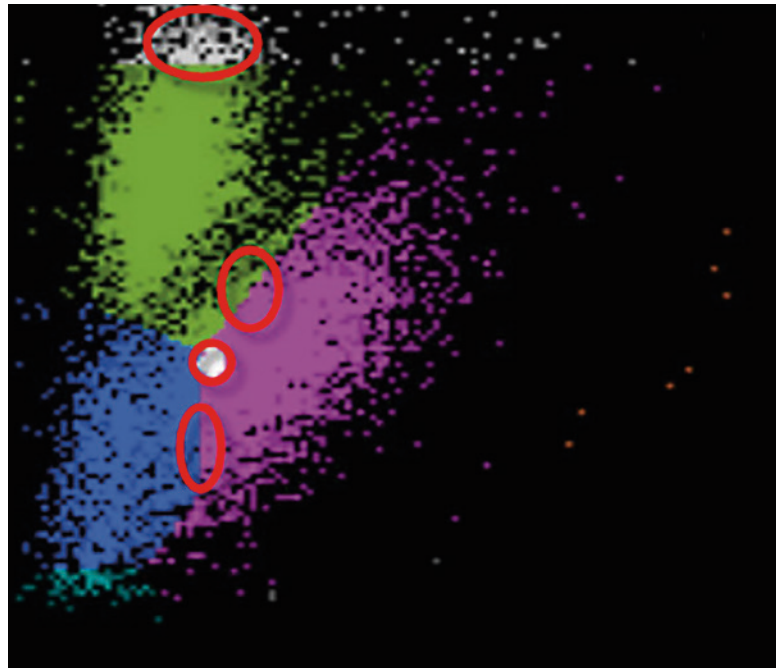
## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- The DxH 500 identified abnormal cell populations.
- PLT “R” due to Cellular Interference in WBC diff scatterplot, below the Lymphocytes and correlates to the NRBC observed.
- Morphology: Abnormal granulocytic cells observed. Platelet estimate agrees with platelet results.
- This case illustrates a severe left shift with anemia, hypochromia.

## CASE 4 – LEUKOCYTOSIS, MONOCYTOSIS

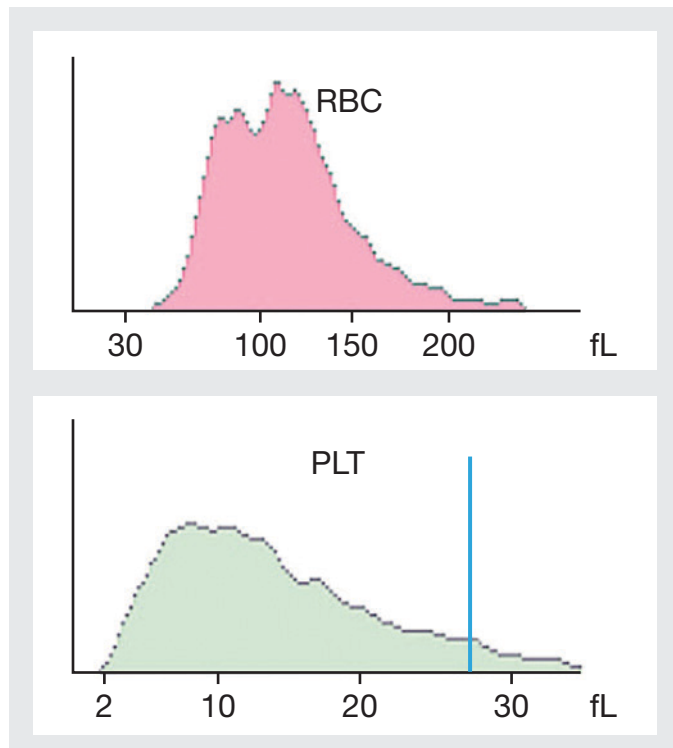
- Abnormal Diff, NE/LY Overlap, MO/NE Overlap, Large Cells, BA Interference, PLT2: Debris; Differential flagged “R”, BA %/# incomplete, PLT flagged “R”

WBC Differential Results			
WBC	12.2	h	$\times 10^3/\mu\text{L}$
LY	17.8	RI	%
MO	45.2	RH	%
NE	35.5	RL	%
EO	0.1	R	%
BA	.....		%
LY#	2.2	R	$\times 10^3/\mu\text{L}$
MO#	5.5	RH	$\times 10^3/\mu\text{L}$
NE#	4.3	R	$\times 10^3/\mu\text{L}$
EO#	0.0	R	$\times 10^3/\mu\text{L}$
BA#	.....		$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot NE/LY Overlap, MO/NE Overlap, Large Cells, BA Interference

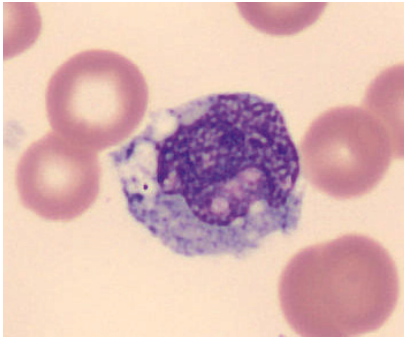
RBC Results			
RBC	1.85	L	$\times 10^6/\mu\text{L}$
HGB	7.1	L	g/dL
HCT	20.8	L	%
MCV	112.7	h	fL
MCH	38.4	H	pg
MCHC	34.1		g/dL
RDW	25.3	H	%
RDW-SD	99.9	H	fL



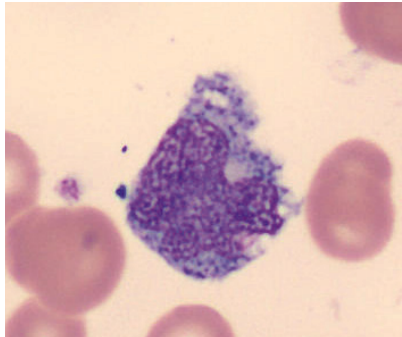
PLT Results			
PLT	89.2	RI	$\times 10^3/\mu\text{L}$
MPV	12.6	RH	fL

The differential scatter plot displays a significant Monocyte population with the Neutrophil population overlapping into the Monocyte, Lymphocyte, and Basophil regions. The cellular overlap initiates the NE/LY Overlap, MO/NE Overlap and BA%/# incomplete messages. The RBC histogram is wide, with two distinct populations of cells, predominantly macrocytic. The PLT histogram, although normal in appearance, exceeds the limits at PLT2, initiating the “PLT 2: Debris” message and PLT “R” flag.

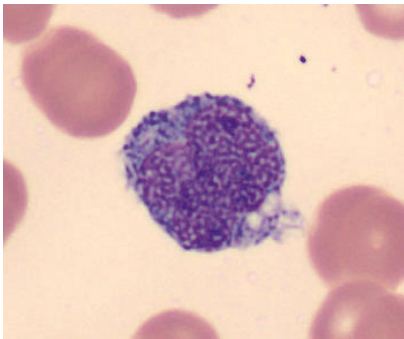
## » Blood Smear (CellaVision DM96)



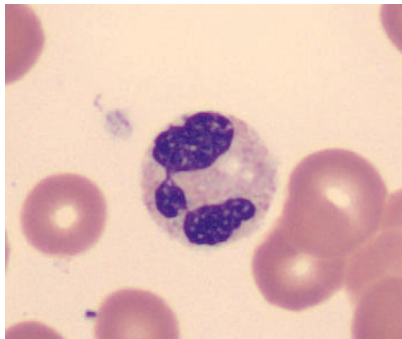
MO with Vacuoles



MO with Vacuoles



MO with Vacuoles



NE



NE

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	1
Metamyelocytes	
Bands	7
Segmented Neutrophils	55
Eosinophils	
Basophils	
Prolymphocytes	
Lymphocytes	4
Atypical Lymphocytes	
Promonocytes	
Monocytes	33
Plasma Cells	
NRBCs	1

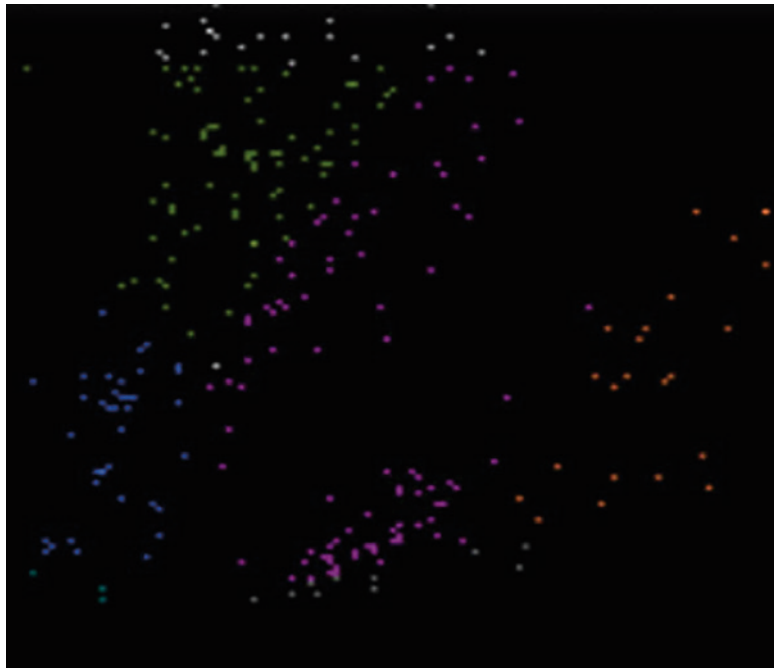
## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- DxH 500 identified the absolute monocytosis as verified by the manual differential results.
- The RBC histogram appears broad, with a large secondary population of macrocytic RBC extending to the right on the RBC histogram at approximately 100 fL, consistent with the elevated RDW and MCV results.
- The PLT results are flagged “R” due to the potential of interference at PLT 2.
- Morphology: 2+ anisocytosis and 1+ macrocytosis with rare large platelets. Vacuoles noted in monocytes and neutrophils.
- This case illustrates an absolute monocytosis, anisocytosis.

## CASE 5 – LEUKOPENIA

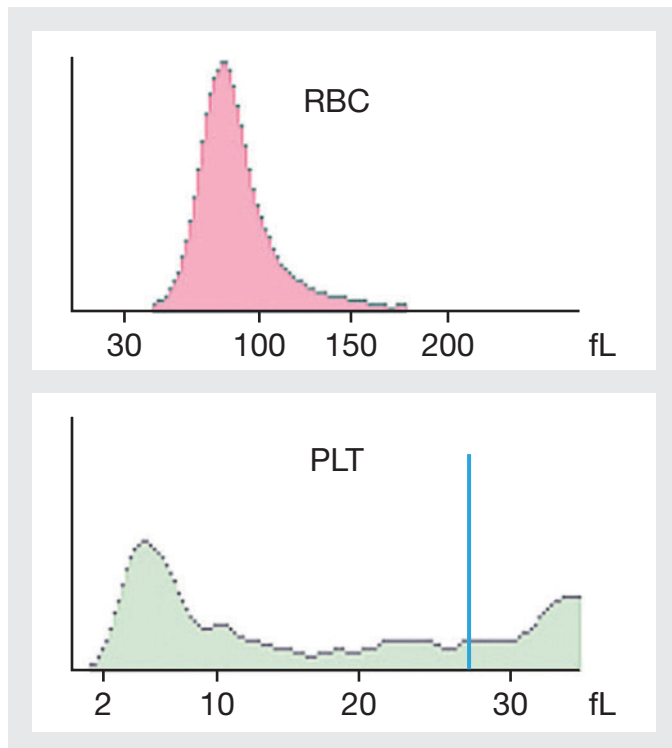
- Abnormal Diff, Large Cells, Low Diff Events, PLT2: Debris; Differential flagged “R”, PLT flagged “R”

WBC Differential Results			
WBC	0.3	L	$\times 10^3/\mu\text{L}$
LY	17.5	RI	%
MO	31.6	RH	%
NE	38.1	RL	%
EO	12.4	RH	%
BA	0.4	R	%
LY#	0.1	RL	$\times 10^3/\mu\text{L}$
MO#	0.1	R	$\times 10^3/\mu\text{L}$
NE#	0.1	RL	$\times 10^3/\mu\text{L}$
EO#	0.0	R	$\times 10^3/\mu\text{L}$
BA#	0.0	R	$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot - Large Cells, Low Diff Events

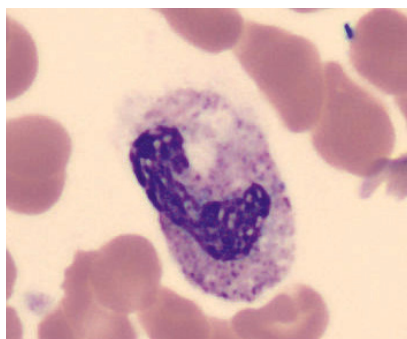
RBC Results			
RBC	3.14	I	$\times 10^6/\mu\text{L}$
HGB	9.5	I	g/dL
HCT	27.2	I	%
MCV	86.5		fL
MCH	30.3		pg
MCHC	34.9		g/dL
RDW	14.5		%
RDW-SD	42.0		fL



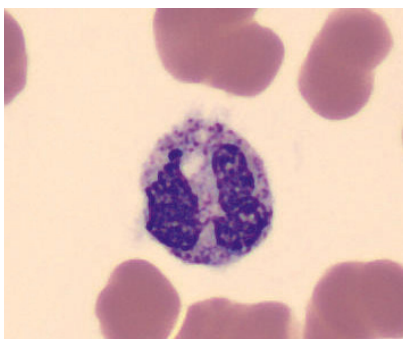
PLT Results			
PLT	24.8	RL	$\times 10^3/\mu\text{L}$
MPV	11.0	R	fL

The differential scatter plot displays very few cellular events correlating with the low WBC count. The differential results are flagged with an “R” is due to Low diff events, Large Cells. The RBC histogram appears normal. The PLT histogram appears abnormal displaying interference, with “PLT 2: Debris” message and PLT “R” flagging of the PLT results.

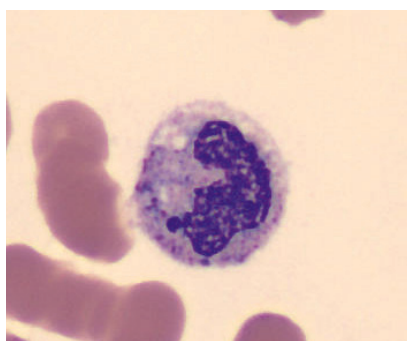
## » Blood Smear (CellaVision DM96)



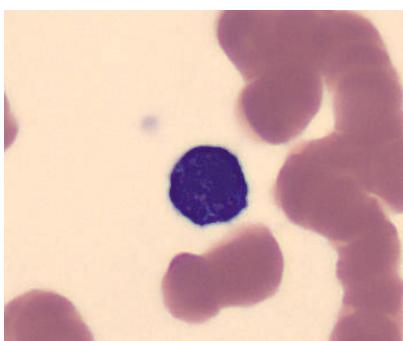
NE Band



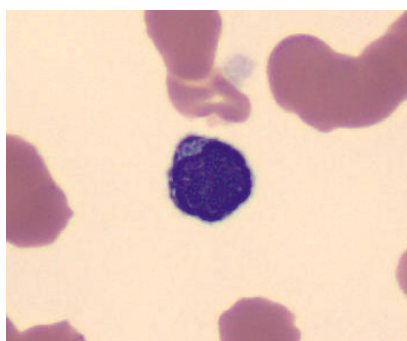
NE



NE



LY



LY

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	17
Segmented Neutrophils	23
Eosinophils	
Basophils	
Prolymphocytes	
Lymphocytes	40
Atypical Lymphocytes	
Promonocytes	
Monocytes	20
Plasma Cells	
NRBCs	

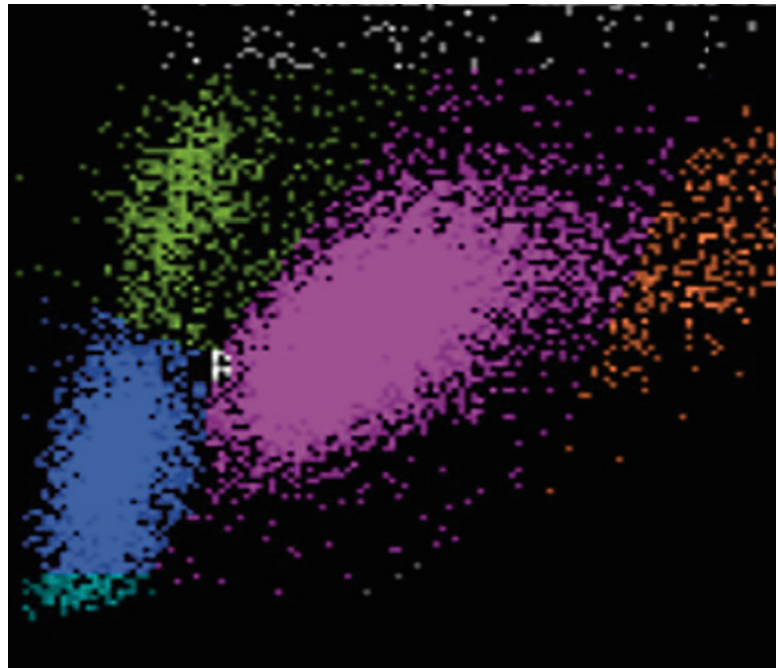
## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- The DxH 500 provided differential results flagged for review due to low number of events.
- The manual differential was performed on 15 cells.
- Morphology: 1+ anisocytosis, microcytosis, rouleaux, platelet estimate approximately 25,000.
- Neutrophils appear hypolobulated.

## CASE 6 – MICROCYTIC RBC, HYPOCHROMIA

- › PLT3: RBC/PLT Overlap; Hct, MCV and related indices flagged “R”, PLT flagged “R”.

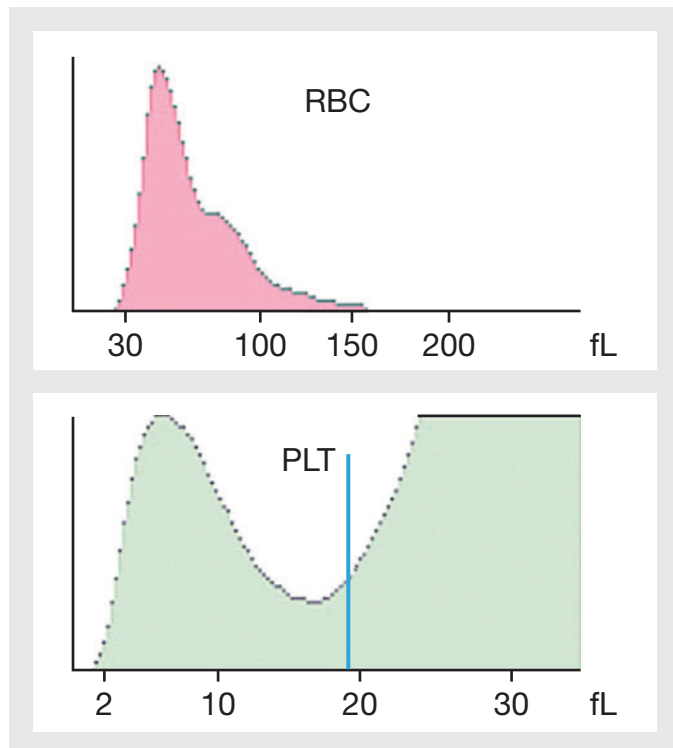
WBC Differential Results			
WBC	12.4	h	$\times 10^3/\mu\text{L}$
LY	23.7	l	%
MO	6.5		%
NE	66.3		%
EO	3.3		%
BA	0.2		%
LY#	2.9		$\times 10^3/\mu\text{L}$
MO#	0.8		$\times 10^3/\mu\text{L}$
NE#	8.2	h	$\times 10^3/\mu\text{L}$
EO#	0.4		$\times 10^3/\mu\text{L}$
BA#	0.0		$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot

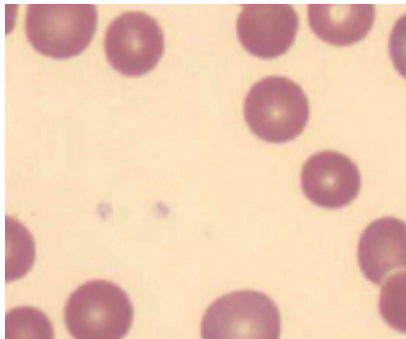
RBC Results			
RBC	4.41		$\times 10^6/\mu\text{L}$
HGB	8.8	L	g/dL
HCT	28.0	RI	%
MCV	63.6	RL	fL
MCH	20.0	L	pg
MCHC	31.4	R	g/dL
RDW	41.0	+	%
RDW-SD	57.2	Rh	fL

PLT Results			
PLT	140.6	Rh	$\times 10^3/\mu\text{L}$
MPV	8.9	R	fL

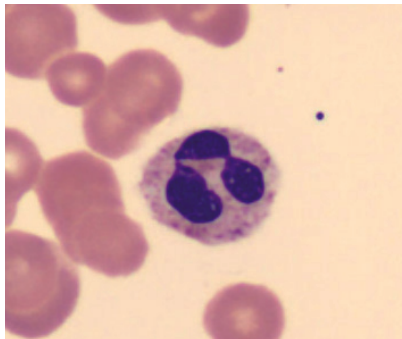


The differential scatter plot appears normal. The RBC histogram is abnormal displaying two populations of cells. The predominant population is microcytic. The RDW parameters are flagged accordingly based on the width of the RBC population on the histogram. The PLT histogram is abnormal with notable interference resulting in the “PLT 3: RBC/PLT Overlap” message and PLT “R” flagging of the PLT results.

» Blood Smear (CellaVision DM96)



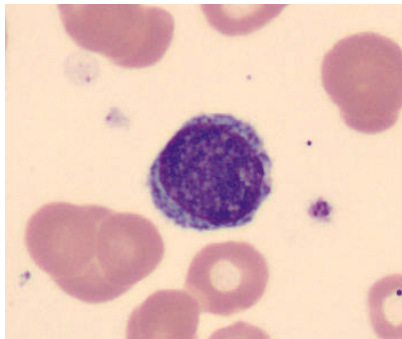
RBC ANISOCYTOSIS



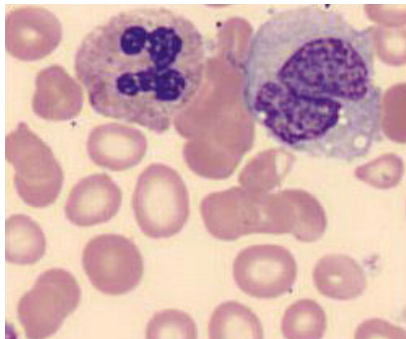
NE



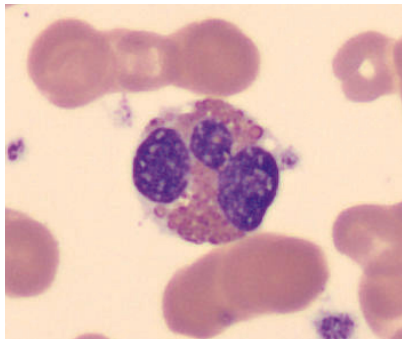
NE



LY



NE, MO



EO

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	6
Segmented Neutrophils	61
Eosinophils	2
Basophils	1
Prolymphocytes	
Lymphocytes	25
Atypical Lymphocytes	1
Promonocytes	
Monocytes	4
Plasma Cells	
NRBCs	

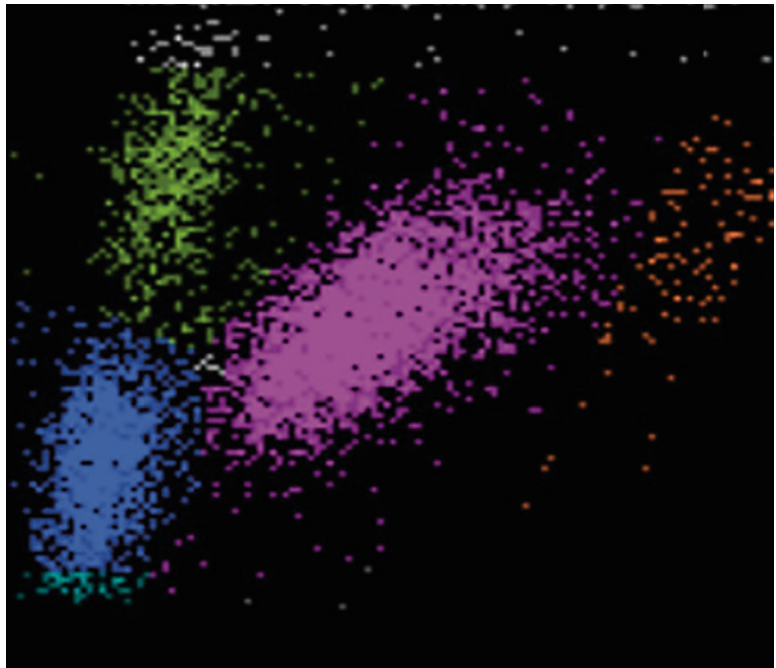
» Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- The DxH 500 identified microcytic RBC on the RBC histogram and the same as interference as “PLT3: RBC/PLT Overlap” on the PLT histogram.
- RBC histogram demonstrates a significant population of microcytic RBC, left of 100 fL. This is consistent with the low MCV and a population of RBC off the right, extending to approximately 120 fL.
- The PLT histogram clearly displays the smaller RBC, as they interfere/overlap with PLTs.
- The RBC and PLT results are flagged for review.
- RBC morphology: 2+ anisocytosis, microcytosis and hypochromia, 1+ poikilocytosis. PLT estimate is normal.
- Instrument results, flags and messages correlate to the review findings obtained on the manual differential.

## CASE 7 – MACROCYTIC RBC

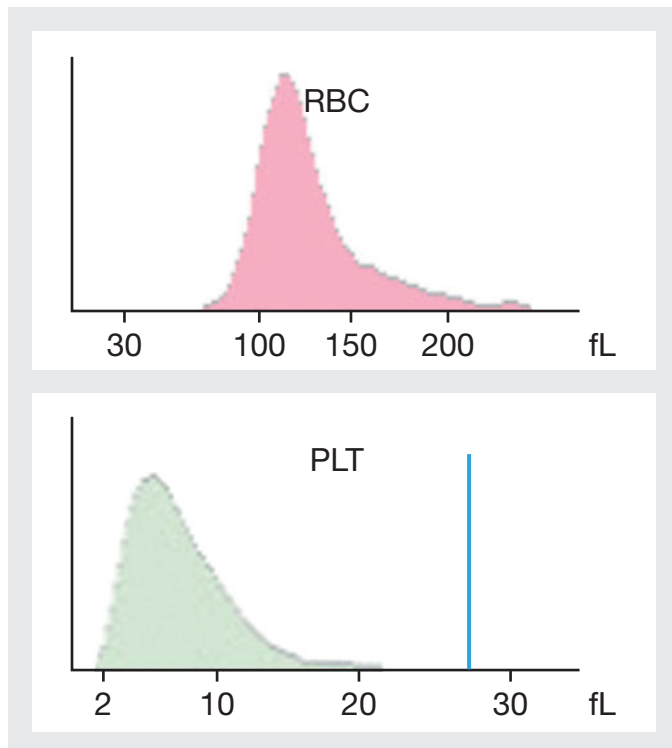
> No instrument flags, codes or messages observed.

WBC Differential Results			
WBC	5.8		$\times 10^3/\mu\text{L}$
LY	25.5		%
MO	10.1	h	%
NE	61.5		%
EO	2.8		%
BA	0.1		%
LY#	1.5		$\times 10^3/\mu\text{L}$
MO#	0.6		$\times 10^3/\mu\text{L}$
NE#	3.6		$\times 10^3/\mu\text{L}$
EO#	0.2		$\times 10^3/\mu\text{L}$
BA#	0.0		$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot

RBC Results			
RBC	2.64	l	$\times 10^6/\mu\text{L}$
HGB	11.2		g/dL
HCT	32.7	l	%
MCV	124.0	H	fL
MCH	42.4	H	pg
MCHC	34.3		g/dL
RDW	12.3		%
RDW-SD	59.8	h	fL

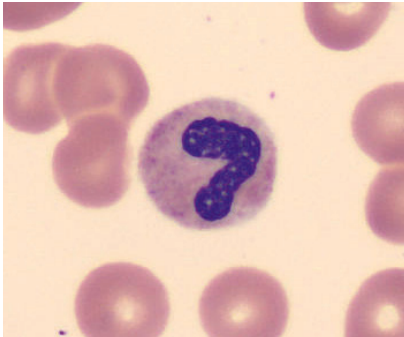


PLT Results			
PLT	198.0		$\times 10^3/\mu\text{L}$
MPV	7.9		fL

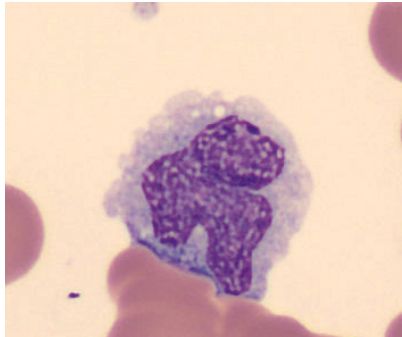
The differential scatter plot appears normal. The RBC histogram appears normal, although macrocytic the mode of the population above 100 fL. The PLT histogram appears normal.



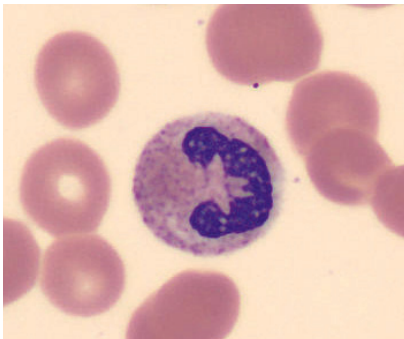
## » Blood Smear (CellaVision DM96)



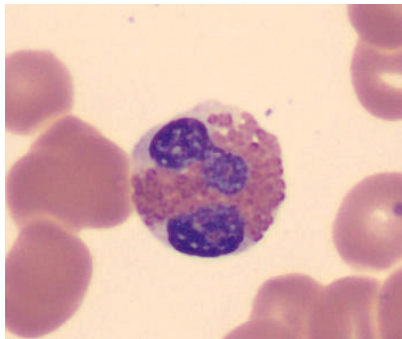
NE



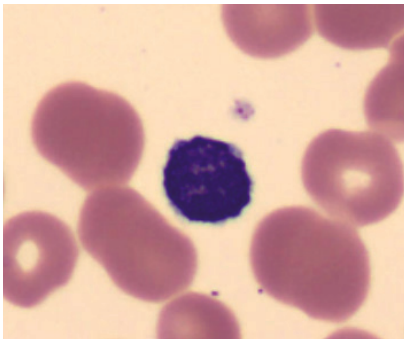
MO



NE



EO



LY

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	3
Segmented Neutrophils	64
Eosinophils	2
Basophils	1
Prolymphocytes	
Lymphocytes	21
Atypical Lymphocytes	1
Promonocytes	
Monocytes	8
Plasma Cells	
NRBCs	

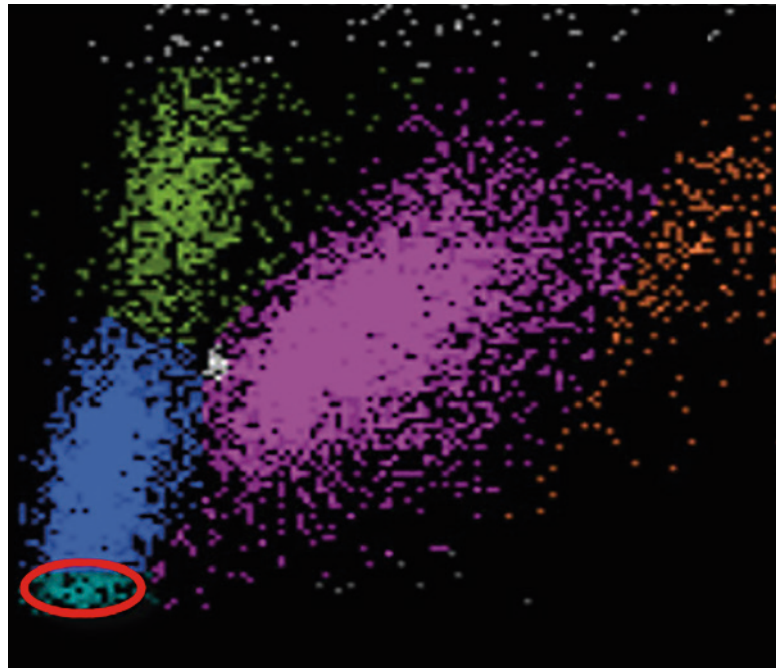
## » Summary Results

- DxH 500 identified macrocytic RBC on the RBC histogram.
- RBC histogram displays RBC population past 100 fL and shifted right, consistent with the elevated MCV.
- The WBC Scatter Plot and the PLT histogram appear normal with no interferences noted.
- Morphology: RBC morphology 1+ anisocytosis and macrocytosis. Platelet estimate low normal.
- Instrument results, flags and messages correlate to the review findings obtained on the manual differential.

# CASE 8 – SICKLE CELL

> Abnormal Diff, Cellular Interference

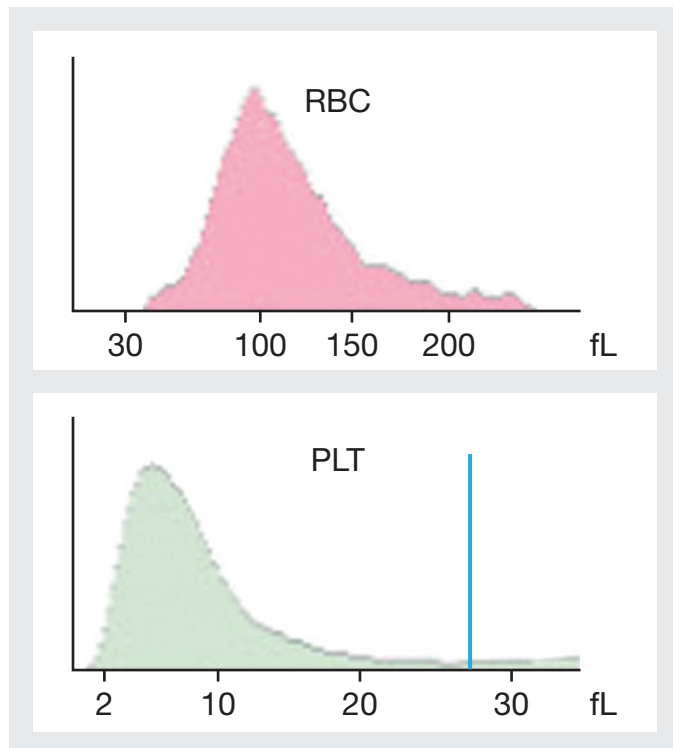
WBC Differential Results			
WBC	8.8	R	$\times 10^3/\mu\text{L}$
LY	27.2	R	%
MO	9.9	R	%
NE	59.1	R	%
EO	3.6	R	%
BA	0.2	R	%
LY#	2.4	R	$\times 10^3/\mu\text{L}$
MO#	0.9	R	$\times 10^3/\mu\text{L}$
NE#	5.2	R	$\times 10^3/\mu\text{L}$
EO#	0.3	R	$\times 10^3/\mu\text{L}$
BA#	0.0	R	$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot with Cellular Interference

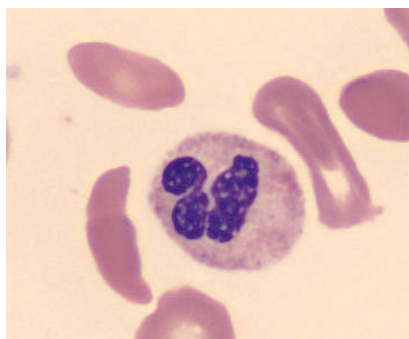
RBC Results			
RBC	1.68	L	$\times 10^6/\mu\text{L}$
HGB	7.1	L	g/dL
HCT	19.5	L	%
MCV	116.1	h	fL
MCH	42.3	H	pg
MCHC	36.4	h	g/dL
RDW	23.9	h	%
RDW-SD	96.5	H	fL

PLT Results			
PLT	211.8	R	$\times 10^3/\mu\text{L}$
MPV	8.8		fL

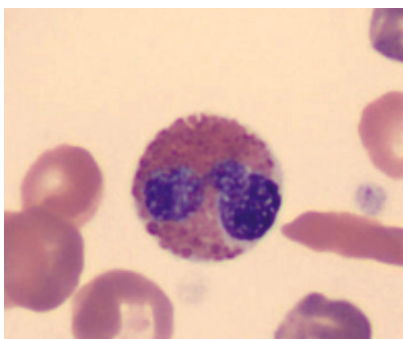


The differential scatter plot displays an extended population of cells from the Lymphocytes into the Cellular Interference region. The RBC histogram appears wide, with larger macrocytic RBC extending right on the histogram. The PLT histogram also appears normal.

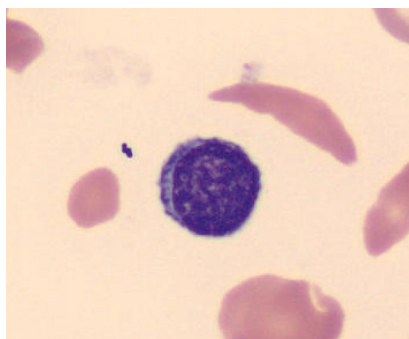
## » Blood Smear (CellaVision DM96)



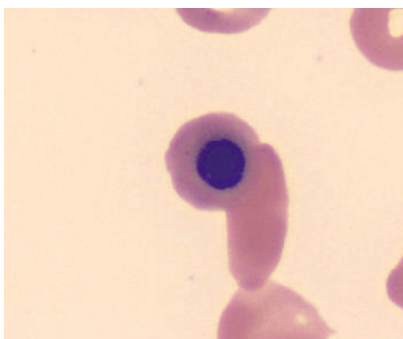
NE



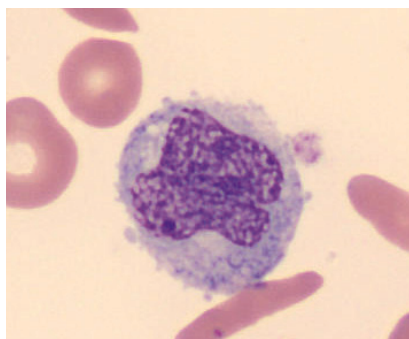
EO



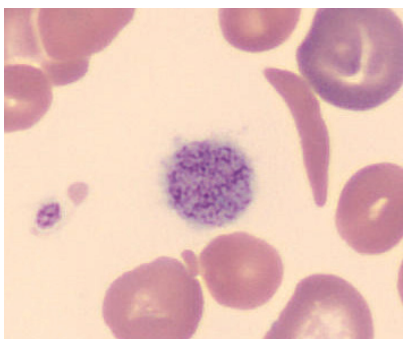
LY



NRBC



MO with PLT



Giant PLT

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	5
Segmented Neutrophils	49
Eosinophils	5
Basophils	1
Prolymphocytes	
Lymphocytes	30
Atypical Lymphocytes	
Promonocytes	
Monocytes	10
Plasma Cells	
NRBCs	7

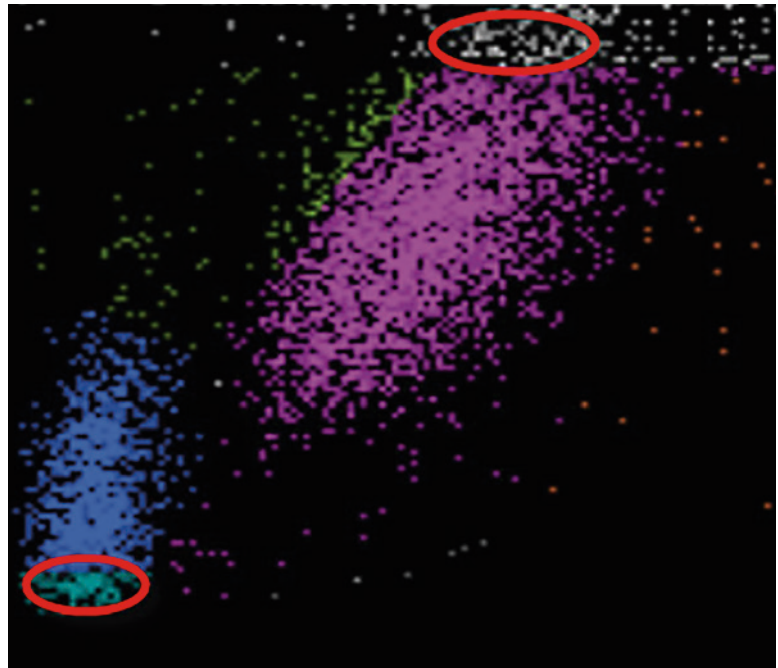
## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- The DxH 500 identified the cellular interference population below the lymphocytes.
- The cellular interference message indicates review for the presence of platelet aggregates, NRBC, sickle cells or lyse-resistant RBC, as verified by the manual differential and smear review.
- The PLT “R” flag is generated by the cellular interference message, indicating a review of the platelet results. The platelet estimate of 225 agrees with the instrument results.
- Morphology: 3+ anisocytosis, macrocytosis, poikilocytosis, sickle cells; 2+ polychromasia.
- An occasional giant platelet and seven 7 NRBC/ 100 WBC were noted on smear review.

# CASE 9 – THROMBOCYTOPENIA, NRBC

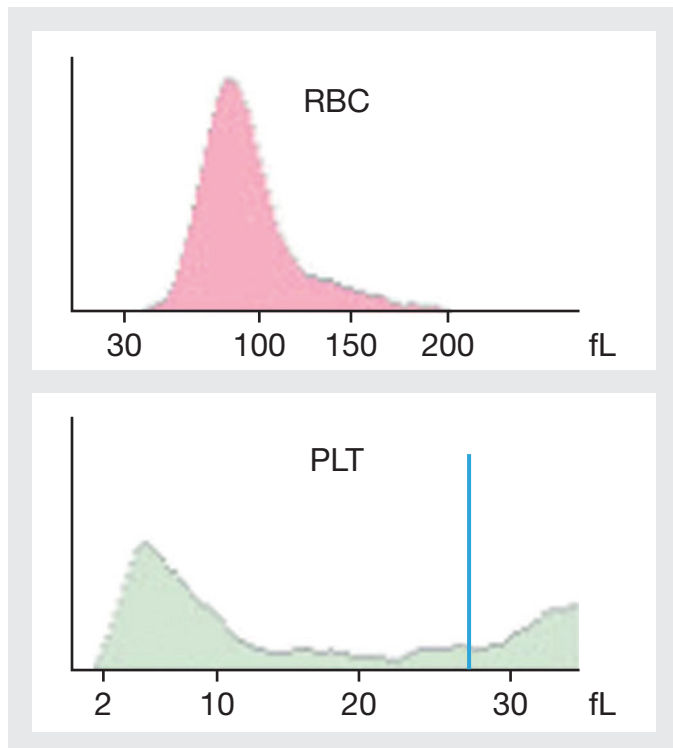
> Abnormal Diff, Cellular Interference, Large Cells

WBC Differential Results			
WBC	4.6	R	$\times 10^3/\mu\text{L}$
LY	28.5	R	%
MO	4.0	R	%
NE	66.8	R	%
EO	0.7	R	%
BA	0.0	R	%
LY#	1.3	R	$\times 10^3/\mu\text{L}$
MO#	0.2	R	$\times 10^3/\mu\text{L}$
NE#	3.1	R	$\times 10^3/\mu\text{L}$
EO#	0.0	R	$\times 10^3/\mu\text{L}$
BA#	0.0	R	$\times 10^3/\mu\text{L}$



DxH Scatter Plot - Cellular Interference, Large Cells

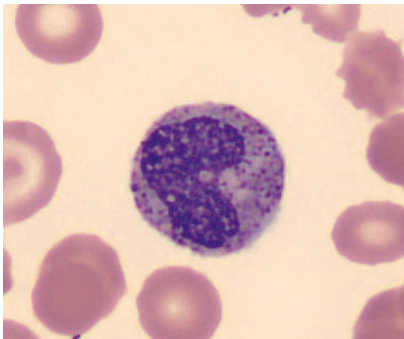
RBC Results			
RBC	2.72	I	$\times 10^6/\mu\text{L}$
HGB	8.7	L	g/dL
HCT	25.5	I	%
MCV	93.9		fL
MCH	32.0		pg
MCHC	34.1		g/dL
RDW	16.7	h	%
RDW-SD	54.5		fL



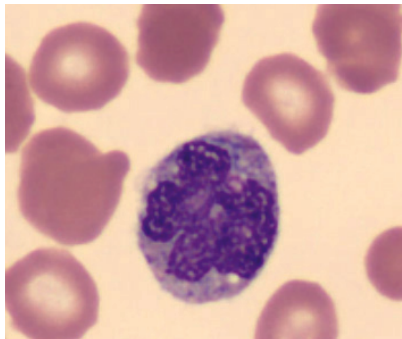
PLT Results			
PLT	18.6	RL	$\times 10^3/\mu\text{L}$
MPV	10.2		fL

The differential scatter plot displays a population of cells extending from the Lymphocytes into the Cellular Interference region and a smaller population extends from the Neutrophils into the Large Cell region. The RBC histogram appears normal. The PLT displays cells at approximately 30 fL. The Cellular Interference message generates the WBC and PLT "R" flags.

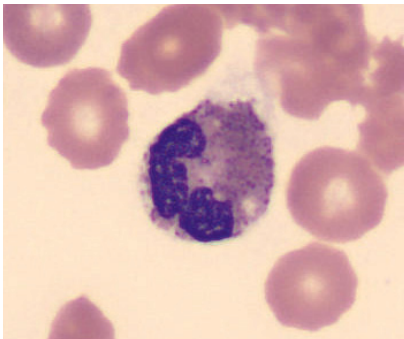
» Blood Smear (CellaVision DM96)



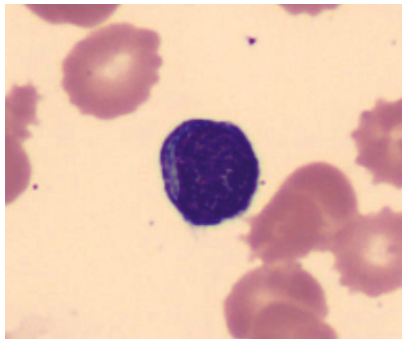
META



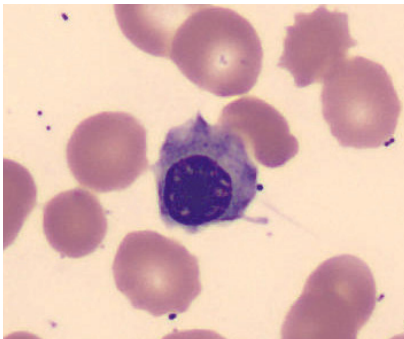
MO



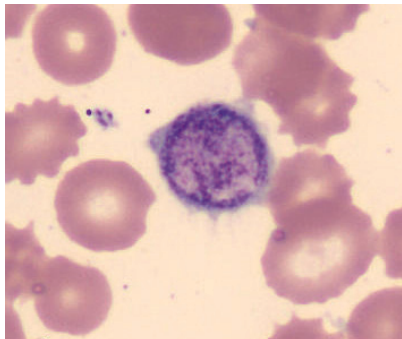
NE



LY



NRBC



Giant PLT

Manual Differential	
Blasts	
Promyelocytes	
Myelocytes	6
Metamyelocytes	18
Bands	55
Segmented Neutrophils	12
Eosinophils	
Basophils	
Prolymphocytes	
Lymphocytes	6
Atypical Lymphocytes	
Promonocytes	
Monocytes	3
Plasma Cells	
NRBCs	43

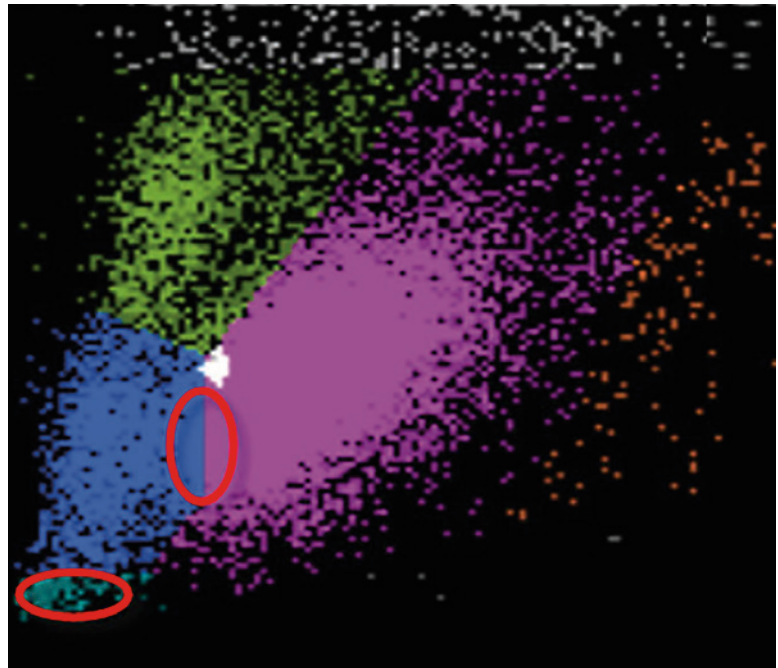
» Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- The DxH 500 identified the cellular interference population indicating review.
- The PLT histogram displays a population at approximately 30fL correlating to the presence of the NRBC and giant platelets seen on the manual differential and smear review.
- Morphology: 2+ anisocytosis, macrocytosis, poikilocytosis, rare target cell, crenated RBC, 43 NRBC/100 WBC; giant platelets present. Platelet estimate correlates to the low platelet results.

# CASE 10 – THROMBOCYTOSIS

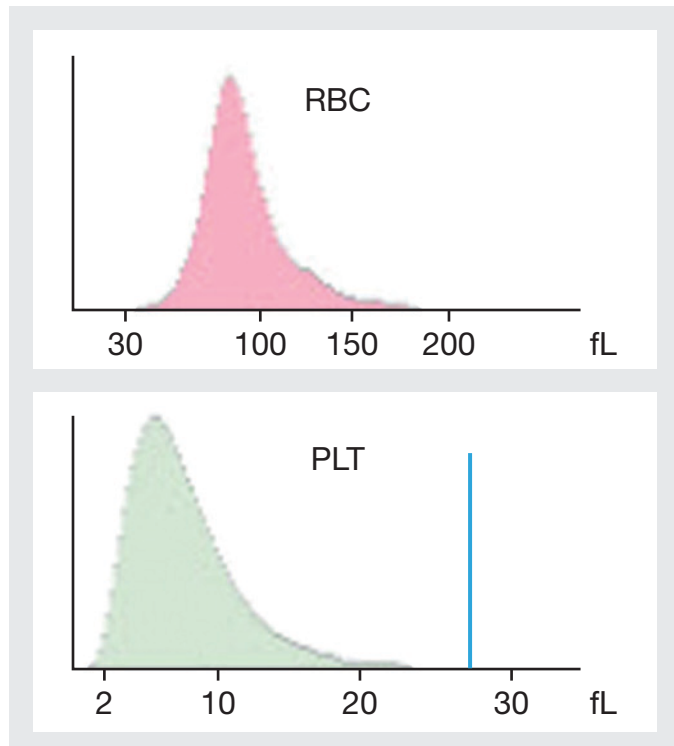
> Abnormal Diff, Cellular Interference, NE/LY Overlap

WBC Differential Results			
WBC	20.1	RH	$\times 10^3/\mu\text{L}$
LY	14.2	RL	%
MO	8.0	R	%
NE	76.2	R	%
EO	0.9	R	%
BA	0.7	R	%
LY#	2.9	R	$\times 10^3/\mu\text{L}$
MO#	1.6	RH	$\times 10^3/\mu\text{L}$
NE#	15.3	RH	$\times 10^3/\mu\text{L}$
EO#	0.2	R	$\times 10^3/\mu\text{L}$
BA#	0.1	R	$\times 10^3/\mu\text{L}$



DxH 500 Scatter Plot - Cellular Interference, NE/LY Overlap

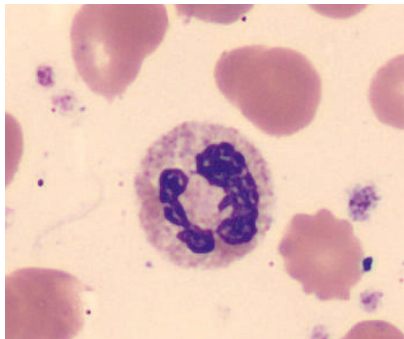
RBC Results			
RBC	3.09	I	$\times 10^6/\mu\text{L}$
HGB	9.3	I	g/dL
HCT	28.4	I	%
MCV	91.9		fL
MCH	30.1		pg
MCHC	32.7		g/dL
RDW	15.3		%
RDW-SD	49.9		fL



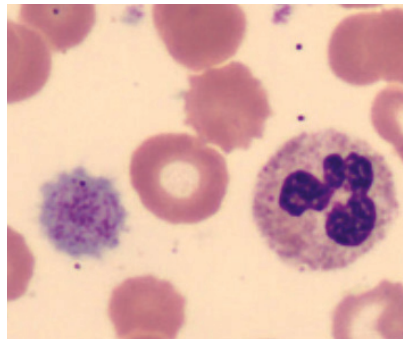
PLT Results			
PLT	1171.0	RH	$\times 10^3/\mu\text{L}$
MPV	7.9		fL

The differential scatter plot displays a population of cells extending from the Lymphocytes into the Cellular Interference region and with an overlap of cells between the Neutrophils and Lymphocytes. The RBC and PLT histograms appear normal. The Cellular Interference message generates the WBC and PLT "R" flags.

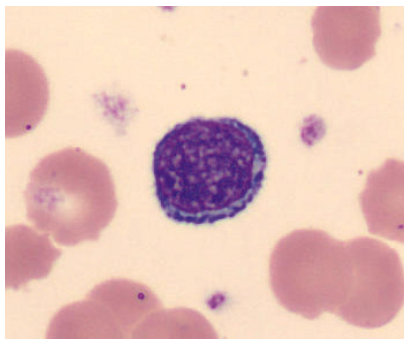
## » Blood Smear (CellaVision DM96)



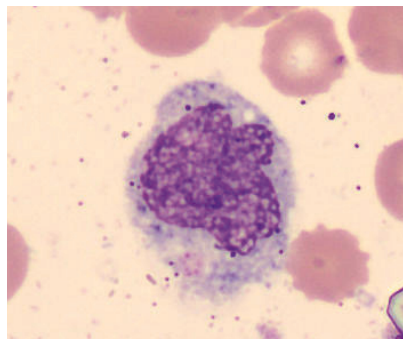
NE



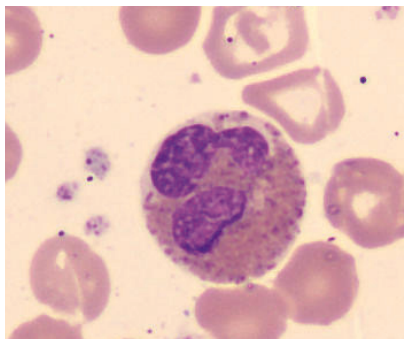
NE with Giant PLT



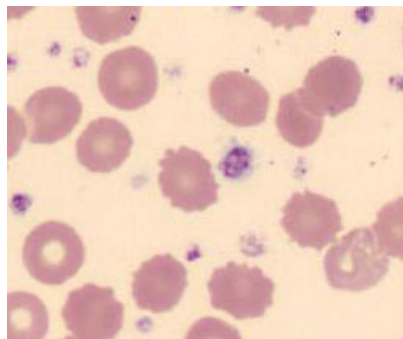
LY



MO



EO



Elevated PLT count with large PLTs

### Manual Differential

Blasts	
Promyelocytes	
Myelocytes	
Metamyelocytes	
Bands	12
Segmented Neutrophils	71
Eosinophils	4
Basophils	
Prolymphocytes	
Lymphocytes	9
Atypical Lymphocytes	
Promonocytes	
Monocytes	4
Plasma Cells	
NRBCs	

## » Summary Results

- Instrument results, flags and messages correlate to the manual differential and smear review.
- DxH 500 identified the cellular interference population below the lymphocytes, indicating possible interference from NRBC, sickle cells, lyse resistant RBC, or clumped/giant platelets.
- The PLT “R” flag generated by the cellular interference message correlates to findings on the manual differential.
- Morphology: 1+ anisocytosis, macrocytosis, 2+ poikilocytosis, platelet estimate 1,200,000 with giant platelets noted.

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**DxH 500**



**DxH 600**



**DxH 800**



**DxH 2401**

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## Reference

1. Coulter, WH. High speed automatic blood cell counter and cell size analyzer. Paper presented at National Electronics Conference, Chicago, IL, 1956; October 3.  
Also: Coulter, W. High speed automatic blood cell counter and cell size analyzer. In Cytometry (3rd edition). Waltham, MA: Elsevier, 1956.

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