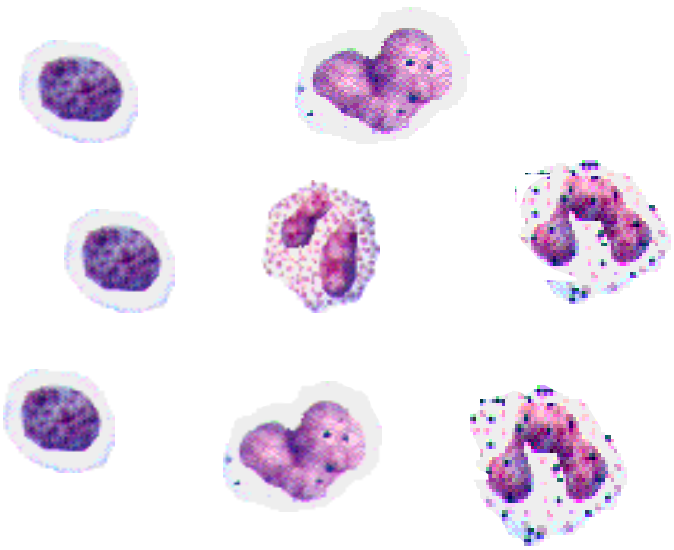
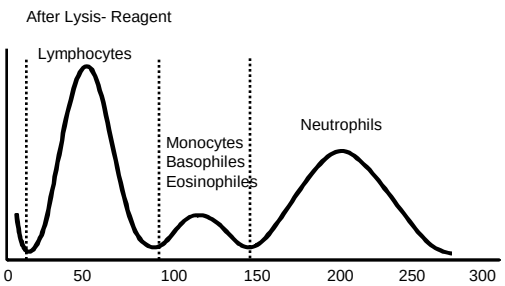
Histogram Interpretation



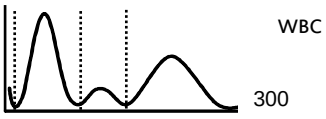


K-Series: Histogram Interpretation

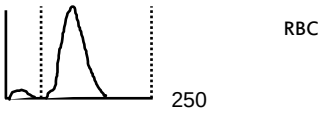
Normal Result

NO. 4
Date: 9/10/95 15:11
MODE: Whole Blood

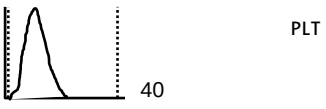
WBC	5,8	x 10^3/µl
RBC	4,84	x10^6/µl
HGB	13,7	g/dl
HCT	42,0	%
MCV	86,8	fl
MCH	28,3	pg
MCHC	32,6	g/dl
PLT	257	x10^3/µl



LYMPH%	31,2	%
MXD%	6,8	%
NEUT%	62,0	%
LYMPH#	1,8	x10^3/µl
MXD#	0,4	x10^3/µl
NEUT#	3,6	x10^3/µl



RDW-SD 40,0 fl



PDW	13,1	fl
MPV	10,4	fl
P-LCR	28,1	%

Parameter of CBC

Leucocyte Histogram

→ Lymphocytes in % and absolut
→ Eo, Mono, Baso in % and absolut
→ Neutrophils in % and absolut

Erythrocyte - Histogram

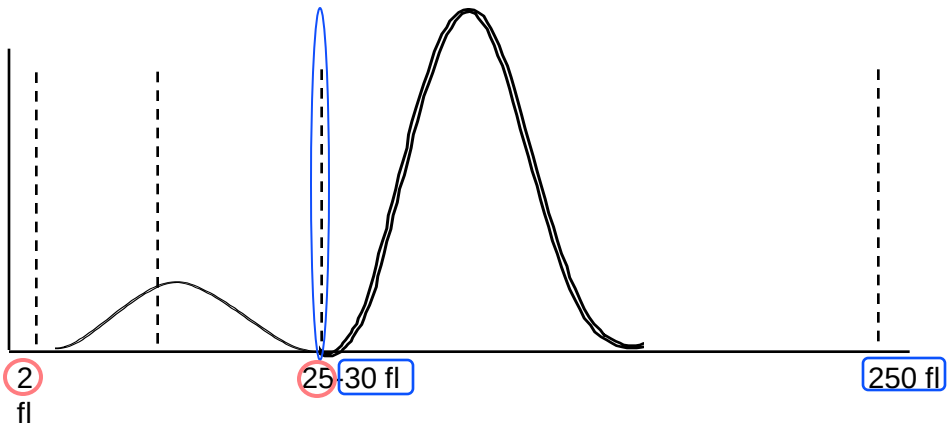
→ RBC Distribution Curve

Thrombocyte Histogram

→PLT Distribution Curve
→Mean PLT Volume
→Share of bigger PLT

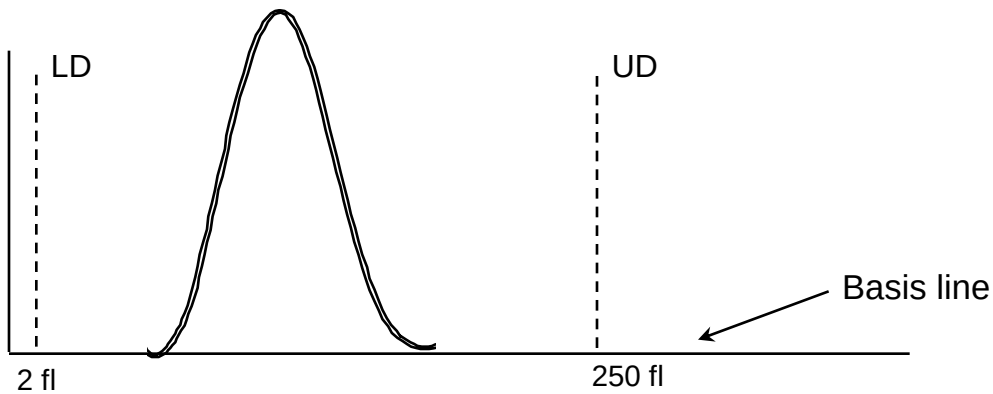
K-Series: Histogram Interpretation

RBC- and PLT-Histograms



- The *two distribution curves* are separated from each other by a moving auto discriminator looking to the Plateau.
- Platelets have a size between 8 and 12 fl and are counted between 2 and 30 fl.
- Erythrocytes have a size of 80-100 fl and are counted between 25 and 250 fl.

LD: Lower Diskriminator
UD: Upper Diskriminator

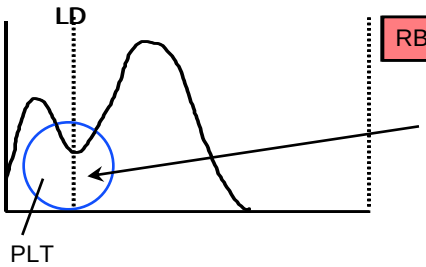


- The Size Distribution Curve should always start on the base line and fall between the lower and the upper discriminator.

K-Series: Histogram Interpretation

Erythrocyte-Histogram Flagging

Mark " **RL** ", abnormal height at lower discriminator

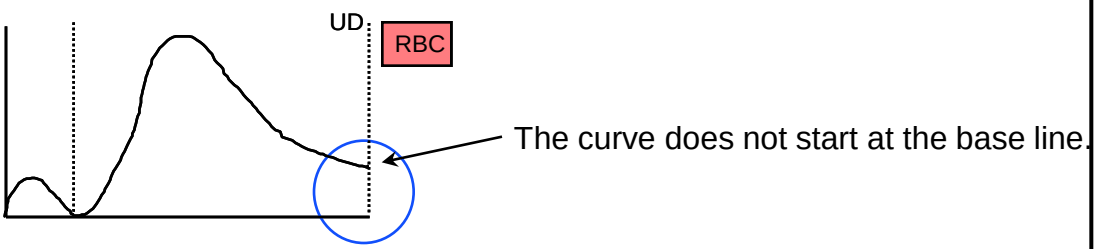


Possible causes:

- Giant Platelets
- Micro-Erythrocytes
- Platelet Clumps

Caution:
All results marked with " RL " should be controlled.

Mark " **RU** ", abnormal height at the upper discriminator.



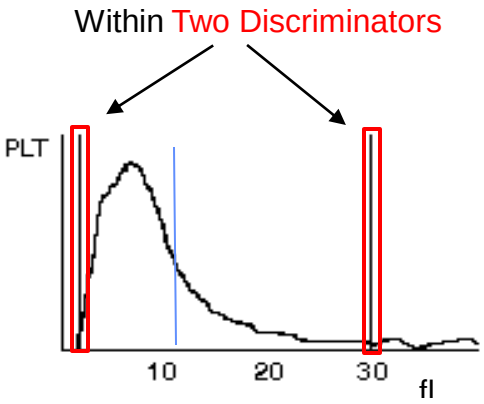
Possible causes:

- Cold Agglutinins (check MCHC > 40 g/dl)
- Erythroblasts / Normoblasts

Caution :
RBC-result and all results marked with " RL " should be controlled.

K-Series: Histogram Interpretation

Thrombocyte-Histogram

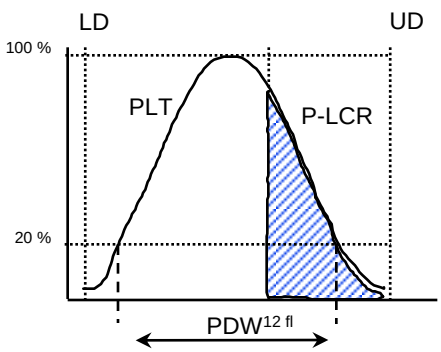


- The histogram should lay within the two discriminators and start and end on the base line.
- PLT counted between 2 fl and 30 fl.
  1 flexible Diskriminator PL 2 to 6 fl.
  1 flexible Diskriminator PU 12-30 fl.
  1 fixed Diskriminator at 12 fl

Parameters of the Thrombocyte histogram

- **MPV**, mean PLT volume
  reference range: **8 - 12 fl**
- **P-LCR**, ratio of large platelets
  Reference range **15 - 35 %**

  Increase could be a sign for:
  - PLT Clumps
  - Giant PLT
  - Microerythrocytes
- **PDW**, platelet distribution width at 20 % of peak height
  Reference range: **9 - 14 fl**
  Increase could be a sign for:
  - PLT Clumps
  - Microerythrocytes
  - Fragments

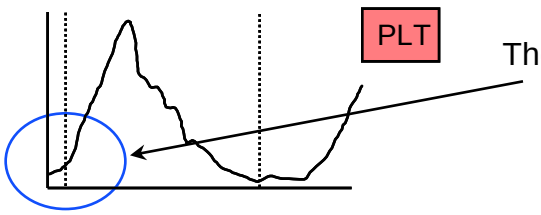


$$\textbf{MPV}\ (\text{fl}) = \frac{\text{Pct}\ (\%)}{\text{PLT}\ (\text{x}\ 10^3/\mu\text{l})}$$

K-Series: Histogram Interpretation

Thrombocyte-Histogram Flagging

Mark " **PL** ", abnormal height at lower discriminator



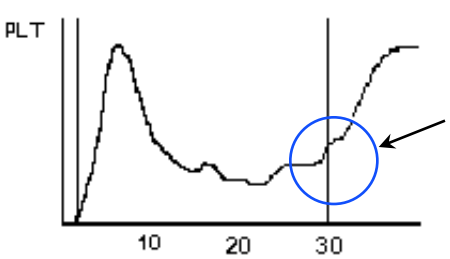
The curve does not start at the base line.

Possible cause:

- High blank value
- Cell fragments

Caution :
Check Blank! Auto Rinse

Mark "**PU**", abnormal height at upper discriminator



The curve does not end on the base line.

Possible Cause :

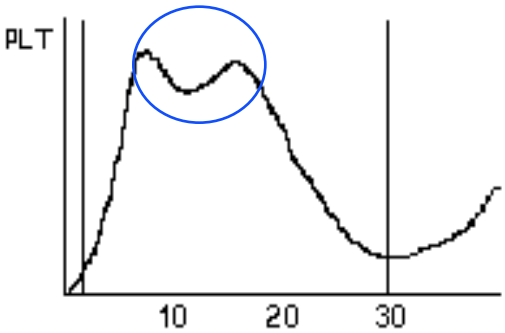
- PLT Clumps
  - EDTA-Incombatibility
  - Clotted sample
- Giant Platelets
- Microerythrocytes

Caution :
Check PLT-Result (and all parameters marked with " PU "! In the event of perform the counting chamber or check PLT via Fonio!

K-Series: Histogram Interpretation

Thrombocyte-Histogram Flagging

Mark "**MP**", Multi Peaks found

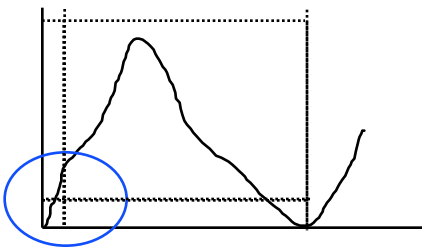


PLT

Possible Cause:

- Platelet transfusion

Mark "**DW**", Distribution With



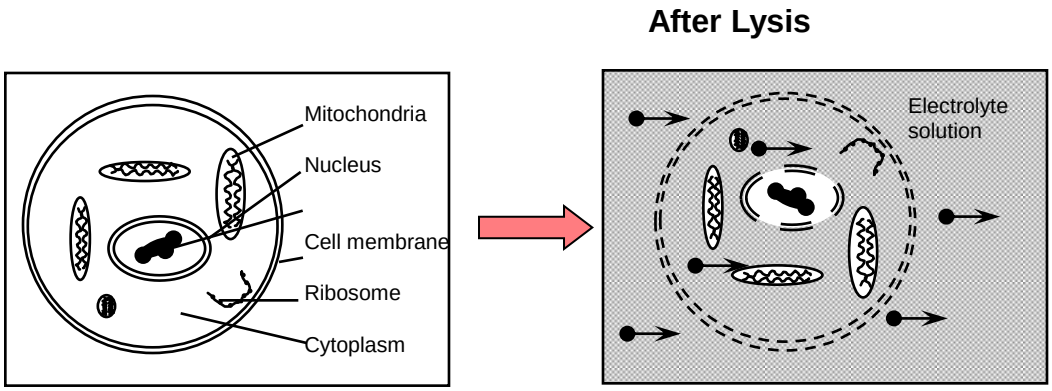
PLT

- The distribution can not be detected because the Histogram does not cross the 20 % limit twice.
- This curve in only an example but could also show another course.
- The overall height of the curve is always 100 %. The width is calculated on the 20 % height of the curve.

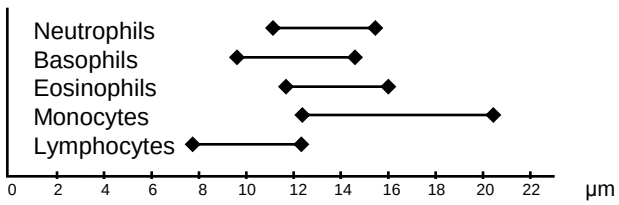
K-Series: Histogram Interpretation

Leukocyte-Histogram

Lyse of RBC and partial lyse of WBC



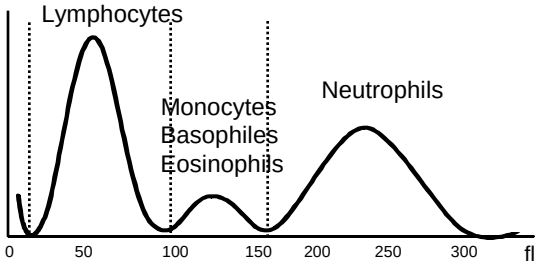
Before adding lysing reagent



Cell diameter in µm

10 - 15	
9 - 14	
11 - 16	
12 - 20	
7 - 12	

After adding lysing reagent

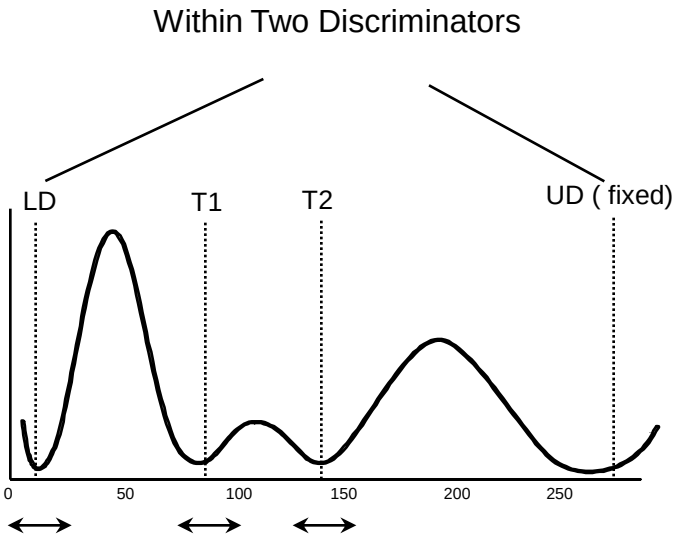


Cell diameter in fl

Lymphocytes	30 - 80
Monocytes	60 - 120
Basophils	70 - 130
Eosinophils	80 - 140
Neutrophils	120 - 250

K-Series: Histogram Interpretation

Leukocyte-Histogram



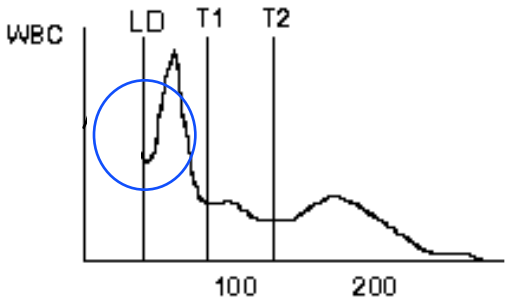
Important :

- The distribution curve should be within the discriminators. The curve should start and end at the basis line.
- The **LD** is flexible, but can not be lower than 30 fl.
- The WBC-channel shows Leukocytes and Thrombocytes (Erythrocytes are lysed).
- The volume of the Thrombocyts is usually between 8 - 12 fl, therefore the LD at the WBC-Histogramm seperates the Leukocytes from the Thrombocytes (Thrombocytes were not counted).

K-Series: Histogram Interpretation

Leukocyte-Histogram Flagging

Flag "**WL**", Curve does not begin at the basis line

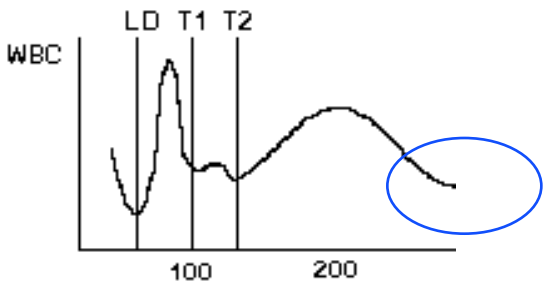


Possible causes :

- PLT Clumps
  - EDTA-Incombatibility
  - coagulated Sample
- high osmotic resistant (Erythrocytes not lysed)
- Erythroblasts
- cold agglutinate

Caution : Check WBC –Result and all parameters marked with "WL"

Flag "**WU**", Curve does not end at the base line.



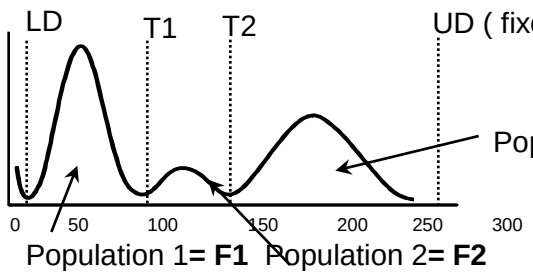
Caution: Check WBC – Result and all parameters marked with "WL"
Dilute sample 1:5 ? (high leukocyte count ?)

K-Series: Histogram Interpretation

Leukocyte-Histogram Flagging

Flag "**T1**" and "**T2**"

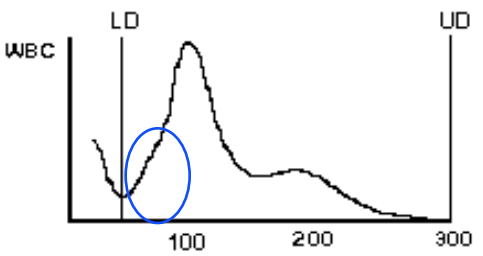
T1 and T2 are valley discriminators defined by the plateau.
This discriminators separates the Leukocytes populations.



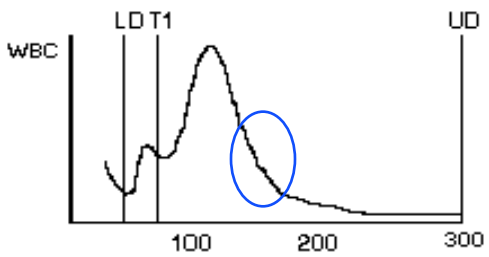
F = Fraction

- The discriminators are flexible and will be set automatically according to the sample.
- In special cases is a separation from the valley discriminators not possible.

T1: T1 could not be detected
No plateau was found.
>**T 1** flag



T2: T1 was detected
but not T2
>**T2** flag



Attention:

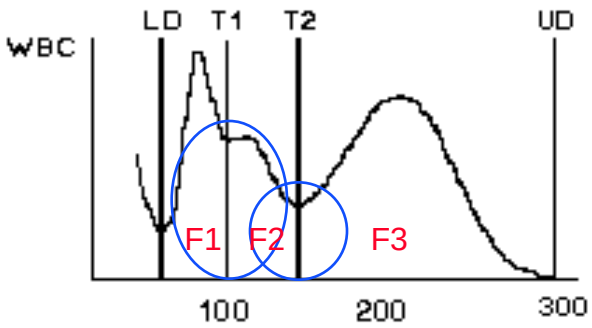
- Confirm the result with the microscope if T1 or T 2 flag was indicated.
- The WBC result will be correct if no flag is indicated. All Leukocytes are counted.

K-Series: Histogram Interpretation

Leukocyte-Histogram Flagging

Flag "**F1**" , "**F2**" and "**F3**"

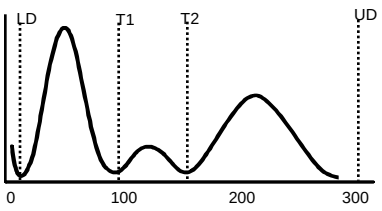
The Histogram of the Leukocytes is limited from the outer discriminators LD and UD.

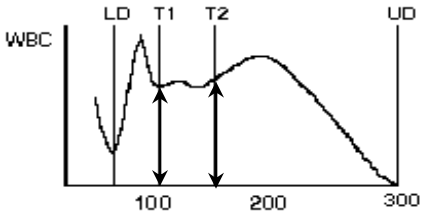


F1: Lymph.
F2: MXD
F3: Neutro.

- All Leukocytes are counted; WBC total is correct. (Assumption: no other flags)
- T 1 and T 2 were detected.
- Conspicuous is:
  The troughs are away from the basis line.

Normal





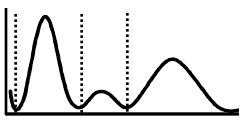
There is a potential of mixing populations.
F 1 and F 2 move together, also F2 and F3.
To get a correct differential it is necassary to do a manual differentiation.

K-Series: Histogram Interpretation

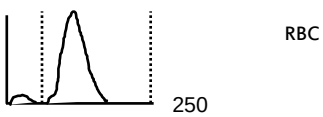
Summery of all flags

NO. 4
DATE: 9/10/95 15:11
MODE: WHOLE BLOOD

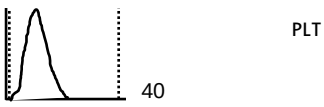
WBC	5,8	x 10^3/µl
RBC	4,84	x10^6/µl
HGB	13,7	g/dl
HCT	42,0	%
MCV	86,8	fl
MCH	28,3	pg
MCHC	32,6	g/dl
PLT	257	x10^3/µl



LYMPH%	31,2	%
MXD%	6,8	%
NEUT%	62,0	%
LYMPH#	1,8	x10^3/µl
MXD#	0,4	x10^3/µl
NEUT#	3,6	x10^3/µl



RDW-SD 40,0 fl



PDW	13,1	fl
MPV	10,4	fl
P-LCR	28,1	%

WL: Abnormal height at lower discriminator of WBC Histogram (LD)
WU: Abnormal height at upper discriminator of WBC Histogram (UD)
T1: Valley 1 not found
T2: Valley 2 not found
F1, F2, F3: Abnormal height at the points **T1** or **T2**; adjacent fractions are marked

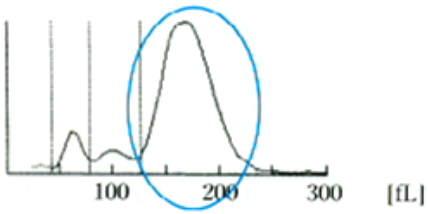
RL: Abnormal height at lower discriminator of RBC Histogram (LD)
RU: Abnormal height at upper discriminator of RBC Histogram (UD)
MP: Multiple peaks: Distinguish ?? of two RBC Populations
DW:The distribution (RDW) can not be detected because the Histogram does not cross the 20 % limit twice.
PL: Abnormal height at lower discriminator of PLT Histogram (LD)
PU: Abnormal height at upper discriminator of PLT Histogram (UD)
MP: Multiple Peaks found
DW:The distribution (PDW) can not be detected because the Histogram does not cross the 20 % limit twice.

The following cases are analysed with the SYSMEX KX-21. Differences of the Histogram-Version are instrument specific and have no analytical influence.

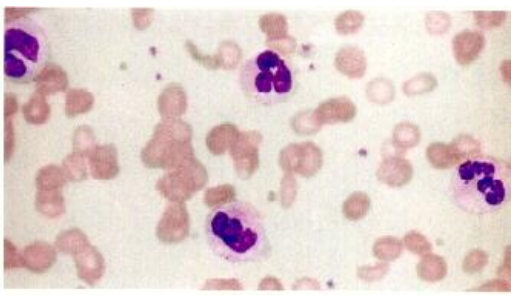
Elevated number of WBC

Neutrophilia

WBC-Histogram



Results		Differential	
WBC	+ 23.8 x 10^9/L	Band	8 %
LYM%	8.1%	Seg	77 %
MXD%	7.9%	Lymph	7 %
NEUT%	84.0%	Mono	7 %
		Eo	1 %
		Baso	0 %



(x 400)

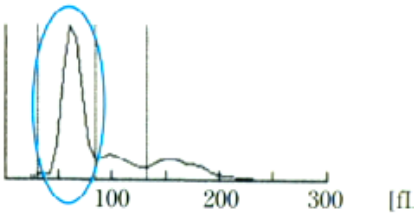
Clinical diagnosis: Neutrophilia

Prominent peak with broad distribution (NEUT%) for large leukocytes.

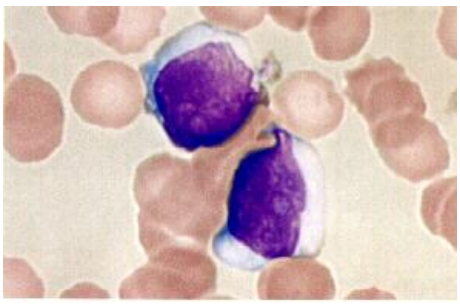
In case of Lymphocytopenia a similar curve is obtained.

Lymphocytosis

WBC-Histogram



Results		Differential	
WBC	7.9 x 10^9/L	Band	4 %
LYM%	+ 64.7%	Seg	20 %
MXD%	15.8%	Lymph	64 %
NEUT%	– 19.5%	Mono	4 %
		Eo	5 %
		Baso	0 %
		Aty-Lym	3 %



(x 1000)

Clinical diagnosis: Lymphocytosis

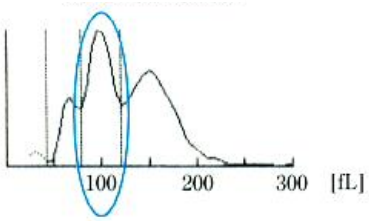
High, pointed peak in lympho area (LYM%).

In case of Neutropenia a similar curve is obtained.

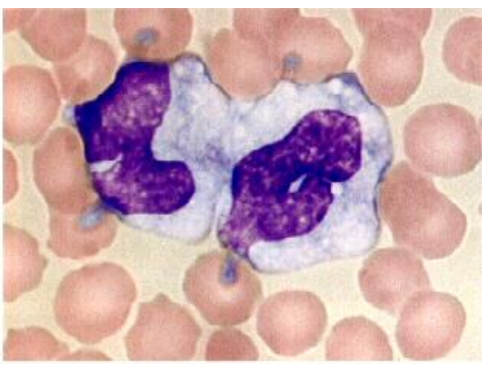
Increase number of WBC

Monocytosis

WBC-Histogram



Results		Differential	
WBC	7.7 x10^9/L F1*	Stab	8 %
LYM%	13.2% F2 *	Seg	37 %
MXD%	37.7%	Lymph	17 %
NEUT%	49.1%	Mono	35 %
		Eo	1 %
		Baso	0 %
		Met	1 %
		Aty-Lym	1 %



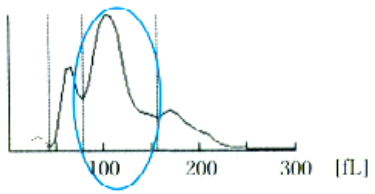
(x 1000)

Clinical diagnosis: Monocytosis

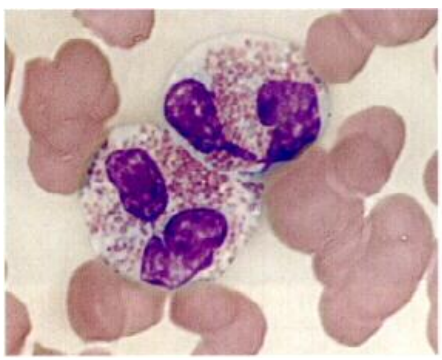
Monocytes, which are the largest leukocytes in normal peripheral blood, become smaller than neutrophils under the influence of the lysing reagent. On the histogram, they fall in the middle cell ratio (MXD%) ( ) . Similar patterns can be seen in eosinophilia. These two different clinical entities need to be differentiated from each other by manual differential.

Eosinophilia

WBC-Histogram



Results		Differential	
WBC	4.3 x 10^9/L	Stab	1 %
LYM%	18,3%	Seg	19 %
MXD%	+ 62,2%	Lymph	20 %
NEUT%	– 19.5%	Mono	9 %
		Eo	47 %
		Baso	1 %
		My	1 %
		Met	1 %
		Aty-Lym	1 %



(x 1000)

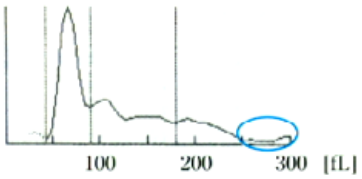
Clinical diagnosis : Eosinophilia

Eosinophils and basophils, which are categorized as granulocytes together with neutrophiles, are smaller than neutrophils due to contraction under the influence of the lysing reagent.
On the histogram, they are located in the middle cell ratio MXD% ( ) where also monocytes are present.
A similar pattern can be seen in monocytosis. Both diseases must be differentiated from each other by manual differential.

WBC Agglutination

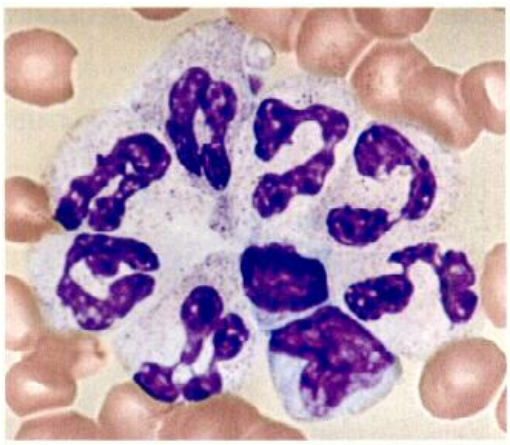
Case 1

WBC-Histogram



Results

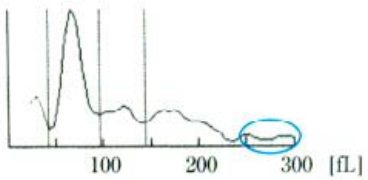
WBC –	2.3 x 10^9/L
LYM%	39.7%
MXD% +	32.2%
NEUT%–	28.1%



(x 1000)

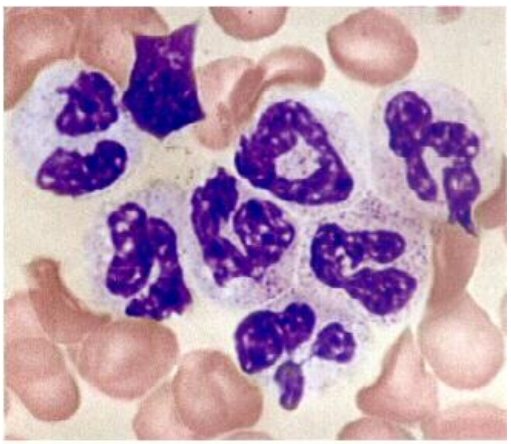
Case 2

WBC-Histogram



Results

WBC –	2.1 x 10^9/L
LYM%	41.9%
MXD%	17.5%
NEUT%–	40.6%

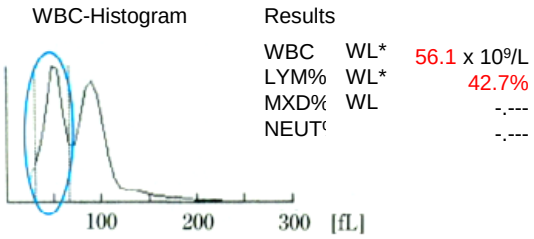


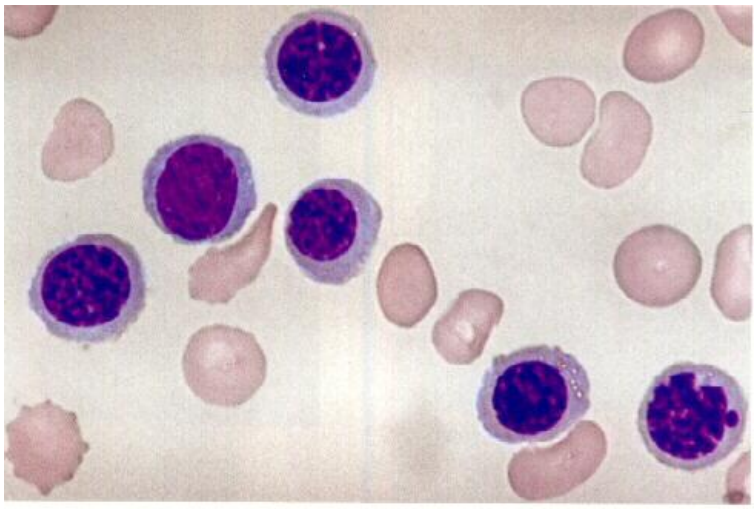
(x 1000)

Case: WBC-Agglutination

This is a case of WBC agglutination, which occurs rather rarely. The histogram does not shown a clear tri-modal pattern, with particles present in the region above 250 fl ( ). The count of leukocytes is likely to be falsely low. *Depending on the nature of leucocytes antibodies*, agglutination may be dissolvable and measurement may become possible upon incubation the at 37 ° C or upon washing the samples with isotonic saline.

Nucleated red blood cells (NRBC)





(x 1000)

Case: Orthochromatic Erythroblasts (NRBC's) at a concentration of 1352/100 WBC

This is a sample with an extreme number of NRBC. The valley between the erythrocytes ghost area and the small leucocytes area exceeds the limit, and **WL flags** are given. NRBC are likely to contribute significantly to the population on the WBC histogram ( ) ; therefore most of them are counted as leukocytes. Measurement of samples having NRBC must be corrected by the following equation:

$$\text{corrected WBC-Count} = \frac{\text{measured WBC-Count} \times 100}{(100 + \text{Count of NRBC's *})}$$

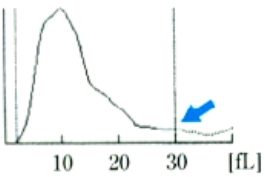
* NRBC-Count: The number of NRBC per 100 leukocytes.

K-Series: Histogram Interpretation Cases

Large Platelets

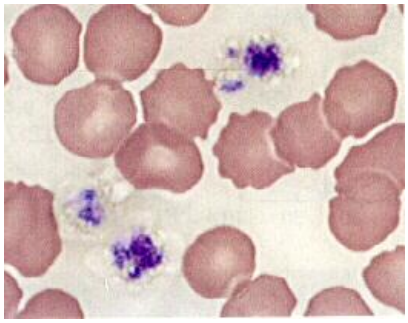
Case 1

PLT-Histogram



Results

PLT		237 x10⁹/L
PDW	+	18.0fl
MPV		12.4fl
P-LCR	+	44.1%



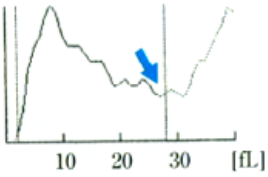
(x 1000)

Case 1: Giant platelets

The abnormally wide distribution on the PLT histogram suggests the appearance of giant platelets. The distribution curve intersects the discriminator line at a low point, which shows that the platelet count has been measured correctly.

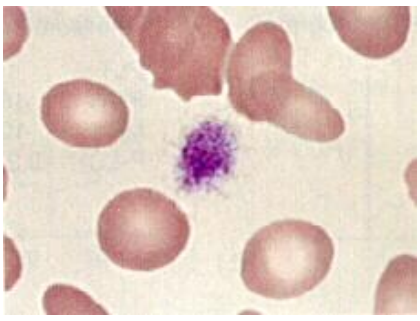
Case 2

PLT-Histogram



Results

PLT	PU	71 x10⁹/L
PDW	DW	---.-fl
MPV	DW	---.-fl
P-LCR	DW	-.---%



(x 1000)

Case 2: large platelets

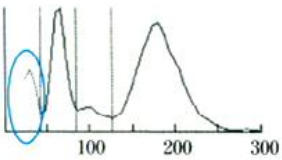
Although the wide distribution on the PLT histogram suggests the appearance of large platelets, the distribution curve intersects the discrimination line at a high point. This result needs to be confirmed by other methods i.e Fonio method or counting chamber.

K-Series: Histogram Interpretation Cases

Platelet Aggregation

Case 1

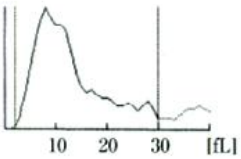
WBC-Histogram



Results

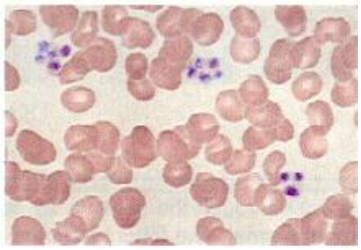
WBC		6.0 x10⁹/L
LYM%		27.5%
MXD%		7.9%
NEUT %		64.4%

PLT-Histogram



Results

PLT		86 x10⁹/L
PDW	+	18.6fl
MPV		12.8fl
P-LCR	+	43.7%



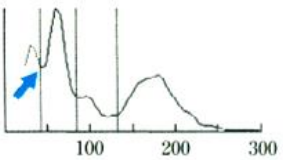
(x 400)

Case 1: Platelet Aggregation

The smear clearly shows that platelets are aggregating. The WBC histogram shows a peak in the ghost area (◯, while the PLT histogram shows a wide distribution. Although these large particles usually affect the leucocyte counts, the leukocytes distribution of case 1 is well separated from the ghost area on the WBC histogram, probably without any effect of small particles in the ghost area. There is no WL Alarm given .

Case 2

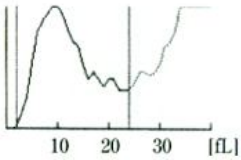
WBC-Histogram



Results

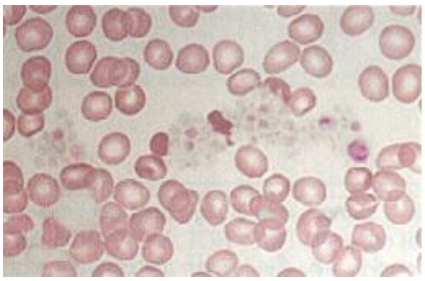
WBC	WL*	6.4 x10⁹/L
LYM%	WL*	41.4%
MXD%	WL*	14.0%
NEUT %	WL*	44.6%

PLT-Histogram



Results

PLT	PU	55 x10⁹/L
PDW	DW	---.-fl
MPV	DW	---.-fl
P-LCR	DW	-.---%



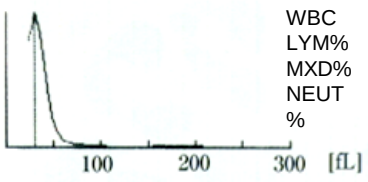
(x 400)

Case 2: Platelet Aggregation

This sample contains larger aggregation clusters as shown in the smear. These clusters are considered affect the leukocyte counts, because the distribution curve on the WBC histogram intersects the discriminator line between the ghost and the Small cell ratio at a high point, and the WL flags are given. The PLT histogram suggests the presence of large particles. Analysis of a fresh blood sample is required to obtain correct platelet values.

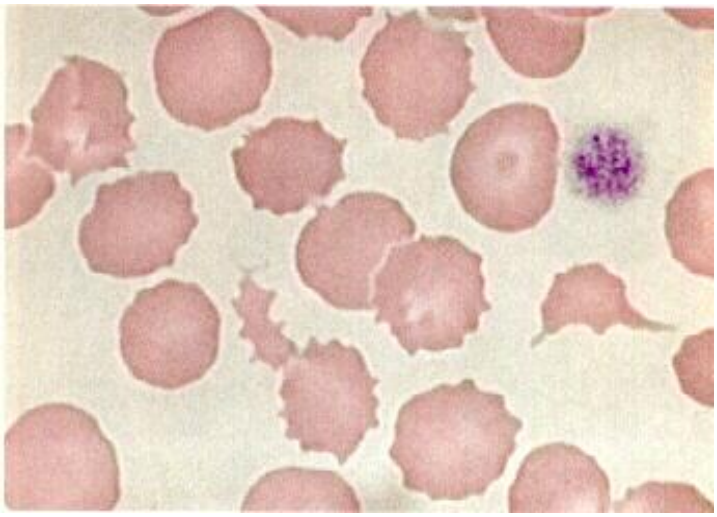
Insufficient Lysing of Erythrocytes

WBC-Histogram



Results

WBC	WL*	49.4 x10^9/L
LYM%	WL	-.---
MXD%	WL	-.---
NEUT %	WL	-.---



(x 1000)

Case: Lyseresistance RBC

The histogram show a pattern typically seen in insufficient lysing of erythrocytes. On the WBC histogram the distribution curve intersects the WBC lower discrimination line at an abnormally high point. The WL flag is output and asterisk marks are put to the leucocyte value, warning of low reliability of the data.
This is frequently seen with blood samples taken from hepatic disease patients or very early newborns. *These problems are solved by diluting the sample or replacing plasma with cellpack (blood cell washing).*

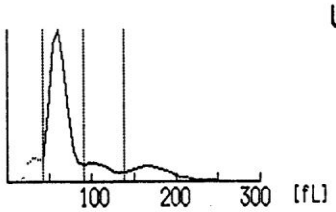
The smear photo shows large platelets and acantocytes, suggesting hepatic diseases.

Erythroblastosis

No. 25
Date 83/03/01 15:30
Mode WB

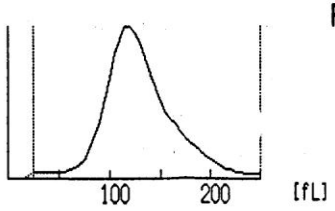
WBC	+	28.5×10³/µL
RBC	-	3.70×10⁶/µL
HGB		14.3g/dL
HCT		45.1%
MCV		+121.9fL
MCH		+ 38.6pg
MCHC		31.7g/dL
PLT	AG	186×10³/µL

WBC



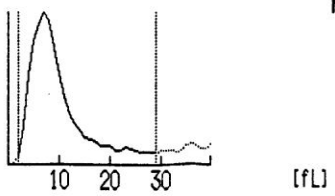
LYM%	+	70.6%
MXD%		12.8%
NEUT%	-	16.6%
LYM#		20.1×10³/µL
MXD#		3.6×10³/µL
NEUT#		4.8×10³/µL

RBC



RDW + 19.2%

PLT



PDW	12.0fL
MPV	10.2fL
P-LCR	26.1%

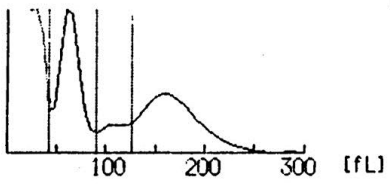
K-Series: Histogram Interpretation

Platelet aggregation

No. 40
Date 83/04/09 10:03
Mode WB

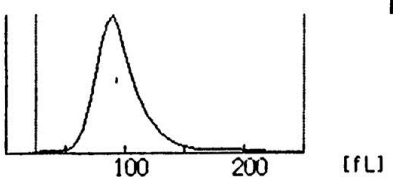
WBC	WL*	10.2×10³/μL	
RBC		5.46×10⁶/μL	
HGB		14.6g/dL	
HCT	+	50.1%	
MCV		91.8fL	
MCH		26.7pg	
MCHC	-	29.1g/dL	
PLT	PL*	36×10³/μL	

WBC



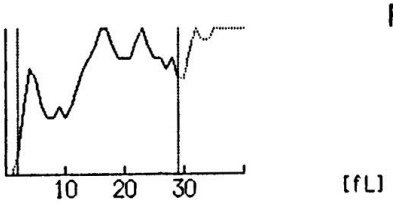
LYM%	WL*	42.4%
MXD%	WL*	11.3%
NEUT%	WL*	46.3%
LYM#	WL*	4.3×10³/μL
MXD#	WL*	1.2×10³/μL
NEUT#	WL*	4.7×10³/μL

RBC



RDW 13.7%

PLT



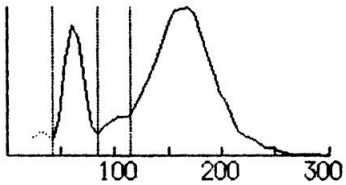
PDW	MP	---.-fL
MPV	PL	---.-fL
P-LCR	PL	---.-%

K-Series: Histogram Interpretation

Fragmented RBCs

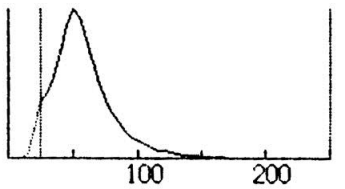
WBC		5.8×10³/μL
RBC	RL*	5.65×10⁶/μL
HGB	-	8.4g/dL
HCT	RL*	32.5%
MCV	RL*	57.5fL
MCH	RL*	14.9pg
MCHC	RL*	25.8g/dL
PLT	PU!	1884×10³/μL

WBC



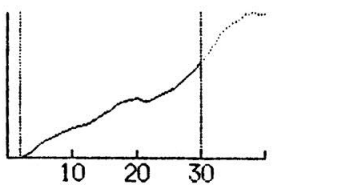
LYM%	21.0%
MXD%	7.2%
NEUT%	71.8%
LYM#	1.2×10³/μL
MXD#	0.4×10³/μL
NEUT#	4.2×10³/μL

RBC



RDW RL* 32.3%

PLT



PDW	DW	---.-fL
MPV	PU	---.-fL
P-LCR	PU	---.-%

K-Series: Histogram Interpretation

Cold Agglutinins