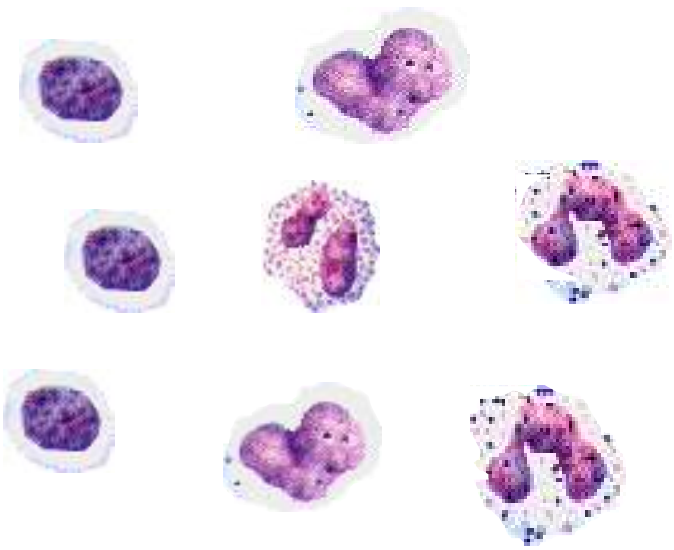
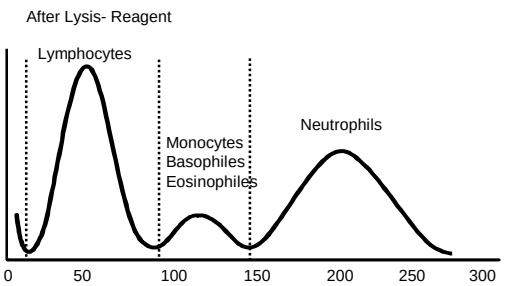
Histogram Interpretation



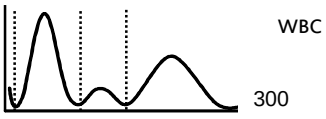


K-Series: Histogram Interpretation

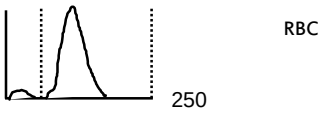
Normal Result

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

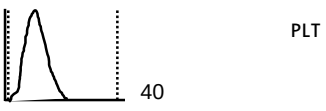
WBC	5,8	x 10^3/µl
RBC	4,84	x10^6/µl
HGB	13,7	g/dl
HCT	42,0	%
MCV	86,8	fl
MCH	28,3	pg
MCHC	32,6	g/dl
PLT	257	x10^3/µl



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

Normal Ranges

White Blood Cell Count

Parameter	Age	Units	SI-Units
WBC	Adults	4-10 x 10^3/µl	x 10^9/l
	Childs	till 12 x 10^3/µl	x 10^9/l
	Newborns	till 15 x 10^3/µl	x 10^9/l
Lymph.	Adults	25-40 %	
	Childs, Newborns	till 70 %	
MXD	Adults	3-13 %	
Neutro.	Adults	50-70 %	
Lymph.	Adults	1-4 x 10^3/µl	x 10^9/l
	Childs	till 5 x 10^3/µl	x 10^9/l
	Newborns	till 6 x 10^3/µl	x 10^9/l
MXD	Adults	0,2-1 x 10^3/µl	x 10^9/l
Neutro.	Adults	2-7 x 10^3/µl	x 10^9/l

Red Blood Cell Count

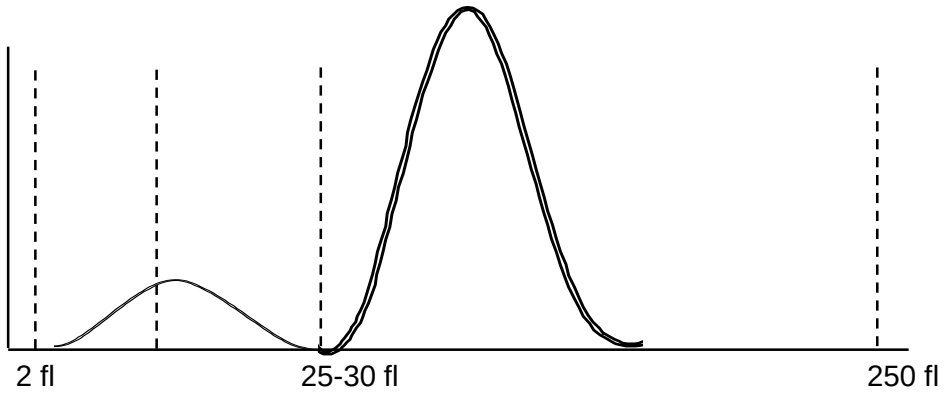
Parameter	Age	Units	SI-Units
RBC	Men	4,6-6,2 x 10^6/µl	x 10^{12}/l
	Women	4,2-5,4 x 10^6/µl	x 10^{12}/l
HGB	Men	14-18 g/dl	8,5-11,0 mmol/l
	Women	12-16 g/dl	7,5-10,0 mmol/l
HCT	Men	43-49 %	0,43-0,49 mmol/l
	Women	36-46 %	0,36-0,46 mmol/l
MCV		85-95 fl	
MCH		27-33 pg	1,68-2,05 fmol
MCHC		32-36 g/dl	19,9-22,4 mmol/l
RDW-SD		37-46 fl (Width in 20% of the Peak hight)	
RDW-CV		11-16 % (calc. width of the 68 % Peak hight)	

Thrombocytes

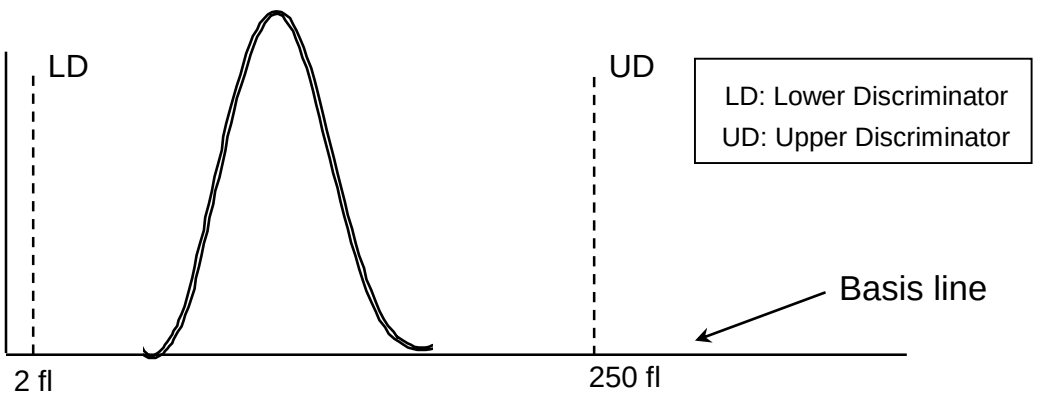
Parameter	Age	Units	SI-Units
PLT		150-400 x 10^3/µl	x 10^9/l
PDW		9-14 fl (Width in 20% of the Peak hight)	
MPV		8-12 fl	
P-LCR		15-35 %	

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.

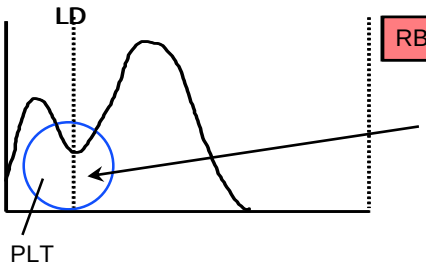


- The Size Distribution Curve should always start on the base line and fall between the lower and the upper discriminator.

K-Series: Histogram Interpretation

Erythrocyte-Histogram Flagging

Mark “ RL “, abnormal height at lower discriminator



The curve does not start at the base line.

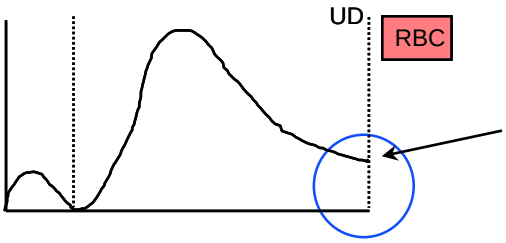
Possible causes:

- Giant Platelets
- Micro-Erythrocytes
- Platelet Clumps

Caution:

All results marked with “ RL “ should be controlled.

Mark “ RU “, abnormal height at the upper discriminator.



The curve does not start at the base line.

Possible causes:

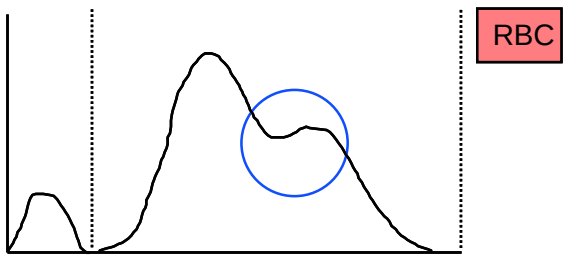
- Cold Agglutinins (check MCHC > 40 g/dl)
- Erythroblasts / Normoblasts

Caution :

RBC-result and all results marked with “ RL “ should be controlled.

Erythrocyte-Histogram Flagging

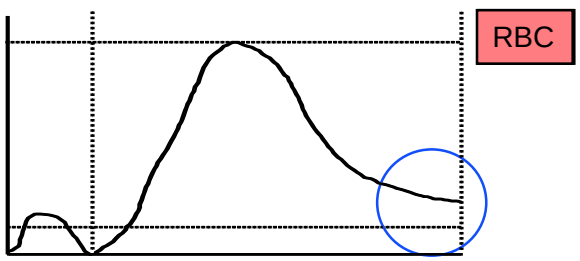
" MP ", multiple peaks found



Possible causes:

- Iron deficiency in therapy
- Infection or Tumor Anemia (visceral iron deficiency)
- Transfusions

"DW ", abnormal histogram distribution

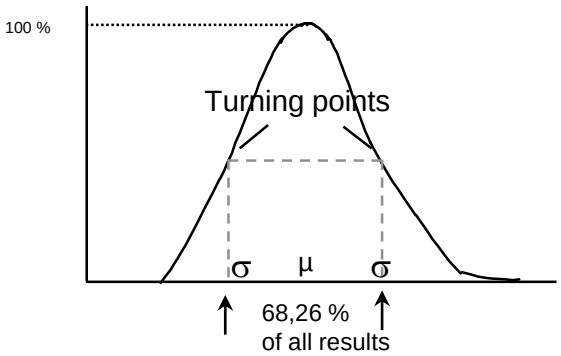


- Distribution curve does not cross 20% level twice.
- The overall height of the curve is always 100 %. The width is calculated on the 20 % height of the curve.
- Hint for extreme Aniso- or. Poikilocytosis.

K-Series: Histogram Interpretation

Erythrocyte-Histogram Distribution width

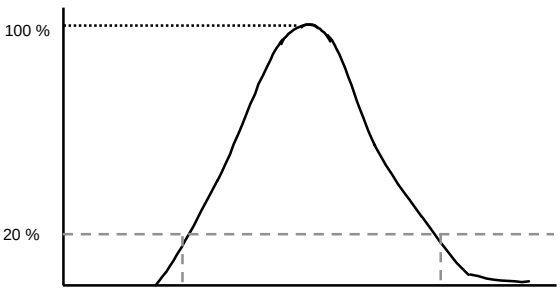
RDW-CV



RDW-CV (%) = 100 x σ/μ

RDW-CV = 11 - 16 %

RDW-SD



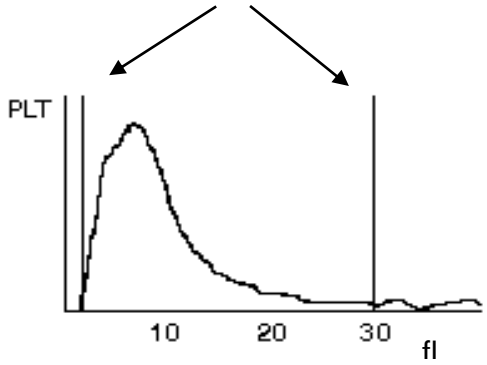
RDW-SD = 37 - 46 fl
Clinical relevant > 60 fl

RBC Distribution Curve as a parameter for anisocytosis

K-Series: Histogram Interpretation

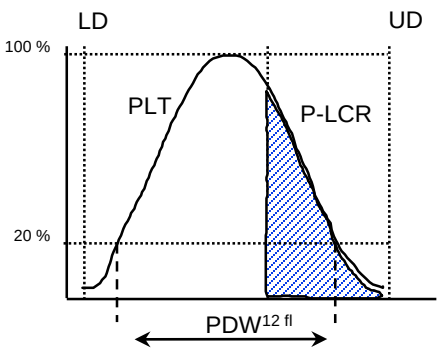
Thrombocyte-Histogram

Within Two Discriminators



- 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 Discriminator PL 2 to 6 fl.
  1 flexible Discriminator PU 12-30 fl.
  1 fixed Discriminator at 12 fl

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



Parameters of the Thrombocyte histogram

- MPV (mean PLT volume) Ref range: 8 - 12 fl
- P-LCR (ratio of large platelets) Ref range: 15 - 35 %
- Increase could be a sign for:
  - PLT Clumps
  - Giant PLT
  - Microerythrocytes

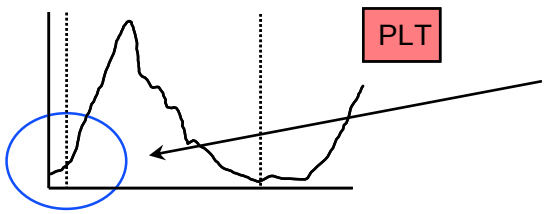
- PDW, (platelet distribution width at 20 % of peak height
  Ref range: 9 - 14 fl

  Increase could be a sign for:
  - PLT Clumps
  - Microerythrocytes
  - Fragments

K-Series: Histogram Interpretation

Thrombocyte-Histogram Flagging

Mark “ PL “, abnormal height at lower discriminator



The curve does not start at the base line.

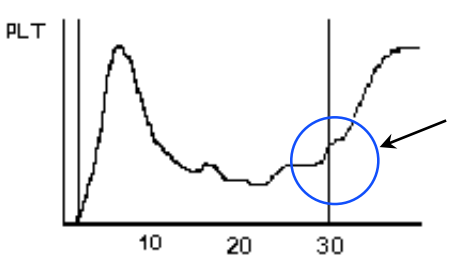
Possible cause:

- 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 :

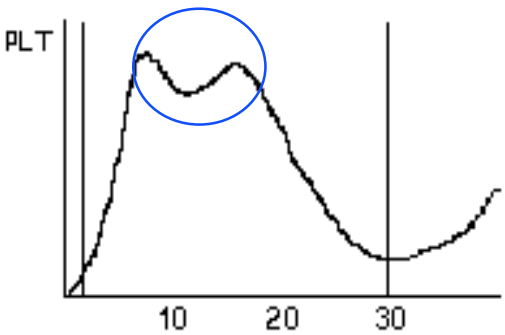
- PLT Clumps
  - EDTA-Incombatibility
  - Clotted sample
- Giant Platelets
- Microerythrocytes

Caution :

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

Thrombocyte-Histogram Flagging

Mark “ MP “, Multi Peaks found

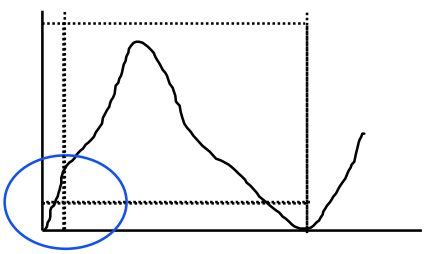


PLT

Possible Cause:

- Platelet transfusion

Mark “ DW “, Distribution With



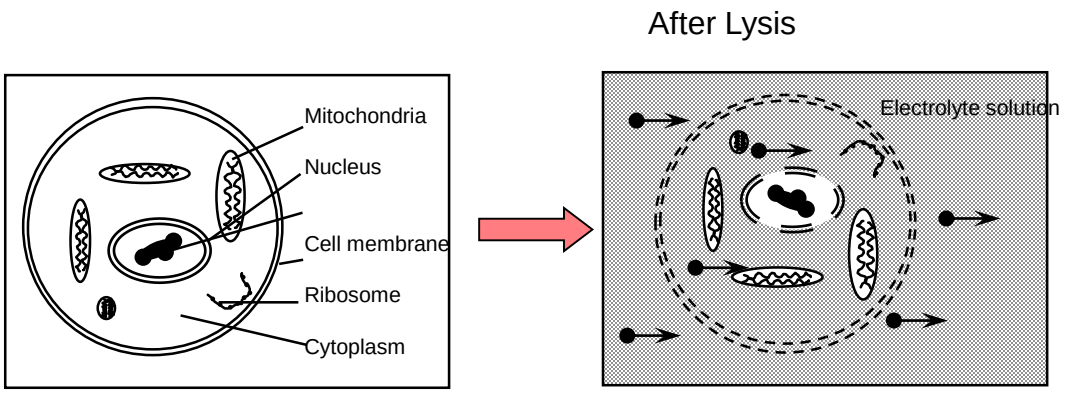
PLT

- 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.

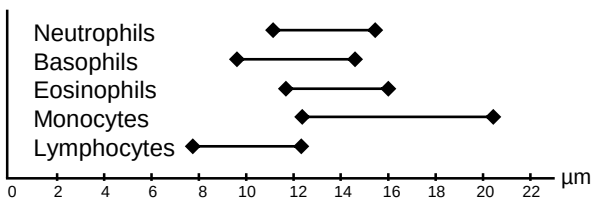
K-Series: Histogram Interpretation

Leukocyte-Histogram

Lyse of RBC and partial lyse of WBC



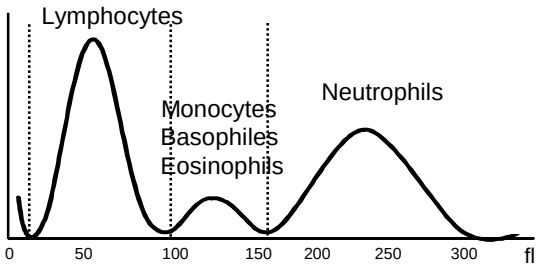
Before adding lysing reagent



Cell diameter in µm

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

After adding lysing reagent

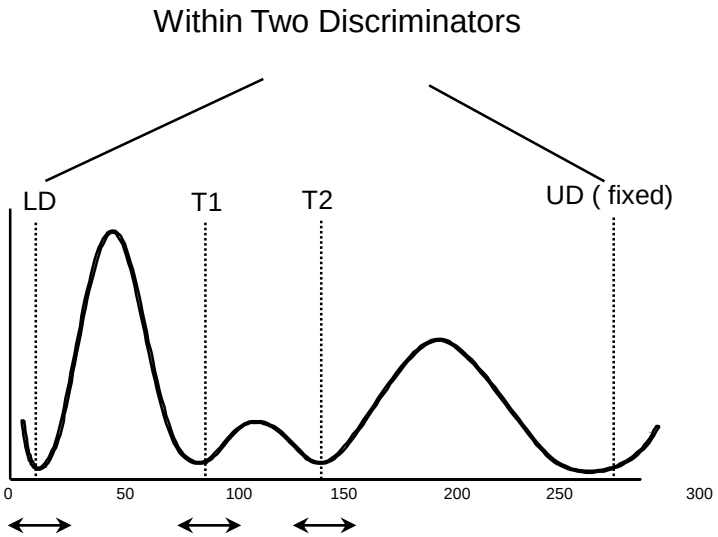


Cell diameter in fl

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

K-Series: Histogram Interpretation

Leukocyte-Histogram

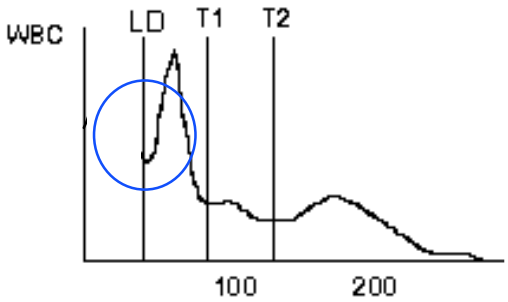


Important :

- 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).

Leukocyte-Histogram Flagging

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

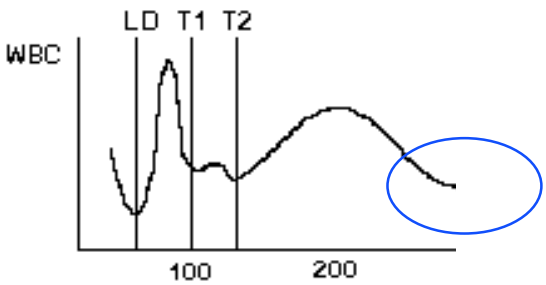


Possible causes :

- 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"

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

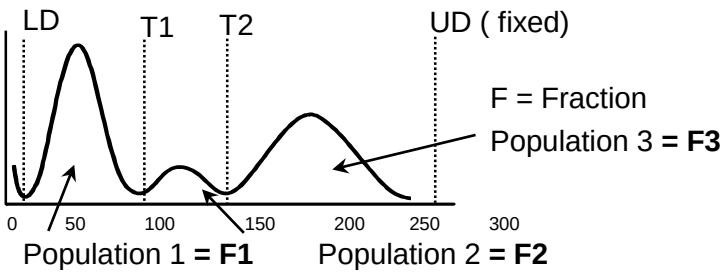


Caution: Check WBC – Result and all parameters marked with "WL"
Dilute sample 1:5 ? (high leukocyte count ?)

Leukocyte-Histogram Flagging

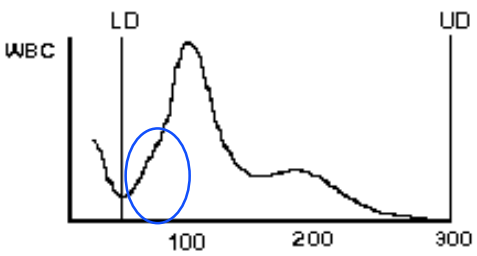
3. Flag “T1” and “T2”

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

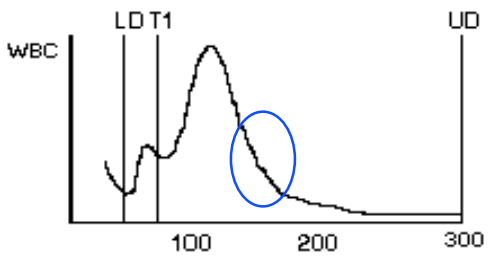


- The discriminators are flexible and will be set automatically according to the sample.
- In special cases is a separation from the valley discriminators not possible.

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



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

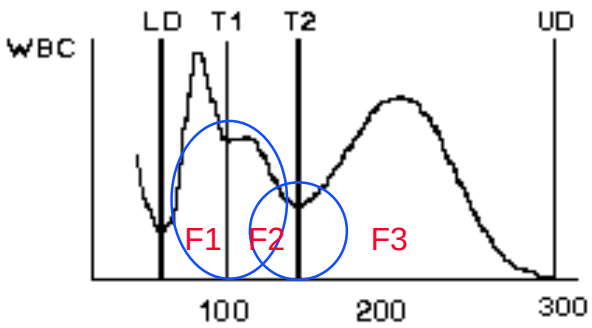


Attention:

- 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.

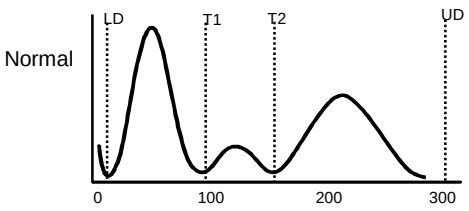
3. Flag “F1” , “F2” and “F3”

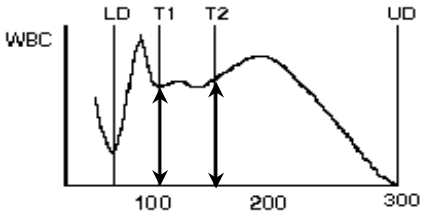
The Histogram of the Leukocytes is limited from the outer discriminators LD and UD.



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.





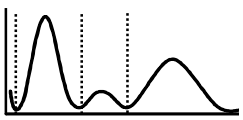
There is a potential of mixing populations.
F 1 and F 2 move together, also F2 and F3.
To get a correct differential it is necassary to do a manual differentiation.

K-Series: Histogram Interpretation

Summery of all flags

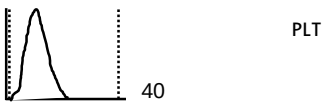
NO. 4
DATE: 9/10/95 15:11
MODE: WHOLE BLOOD

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.

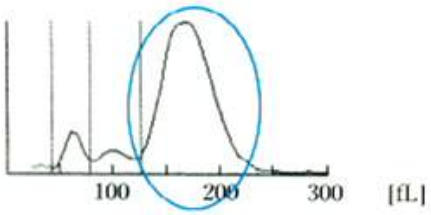
The following cases are analysed with the SYSMEX KX-21.
Differences of the Histogram-Version are instrument specific and have no analytical influence.

K-Series: Histogram Interpretation Cases

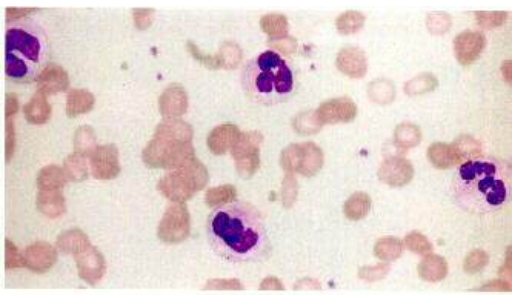
Elevated number of WBC

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)

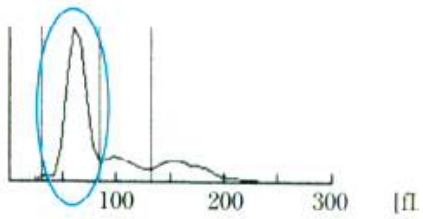
Clinical diagnosis: Neutrophilia

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

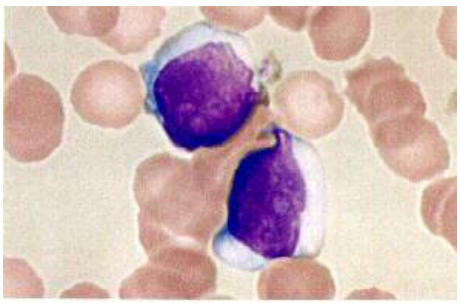
In case of Lymphocytopenia a similar curve is obtained.

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

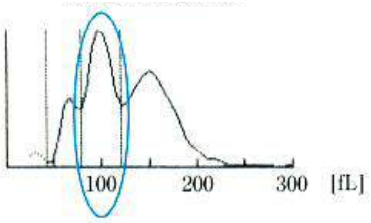
High, pointed peak in lympho area (LYM%).

In case of Neutropenia a similar curve is obtained.

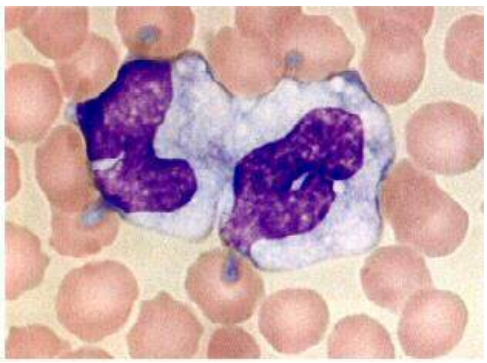
Increase number of WBC

Monocytosis

WBC-Histogram



Results		Differential	
WBC	7.7 x 10^9/L	Stab	8 %
LYM%F1 *	13.2%	Seg	37 %
MXD%F2 *	37.7%	Lymph	17 %
NEUT%	49.1%	Mono	35 %
		Eo	1 %
		Baso	0 %
		Met	1 %
		Aty-Lym	1 %



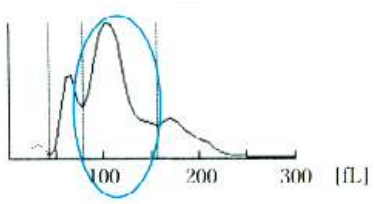
(x 1000)

Clinical diagnosis: Monocytosis

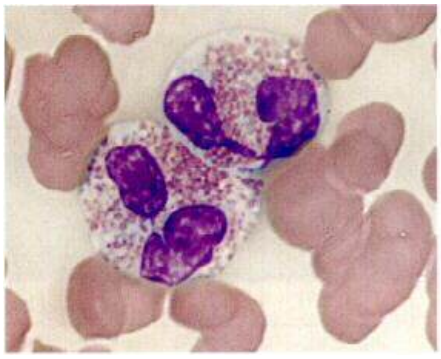
Monocytes, which are the largest leukocytes in normal peripheral blood, become smaller than neutrophils under the influence of the lysing reagent. On the histogram, they fall in the middle cell ratio (MXD%) (⬭) .
Similar patterns can be seen in eosinophilia. These two different clinical entities need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

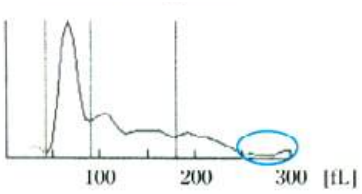
Clinical diagnosis : Eosinophilia

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

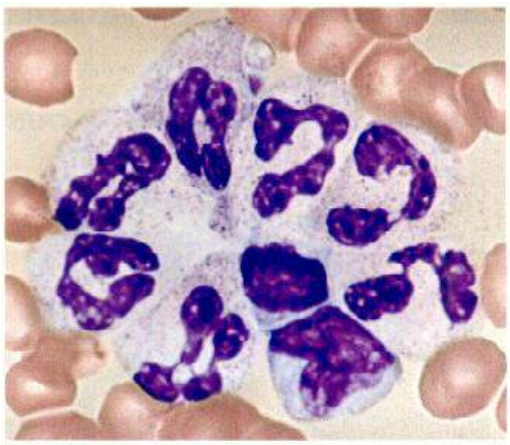
Case 1

WBC-Histogram



Results

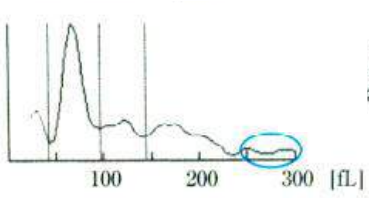
WBC	2.3 x 10^9/L
LYM%	39.7%
MXD%	32.2%
NEUT%	28.1%



(x 1000)

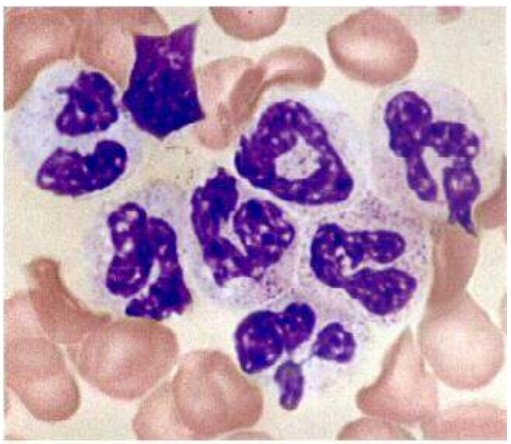
Case 2

WBC-Histogram



Results

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

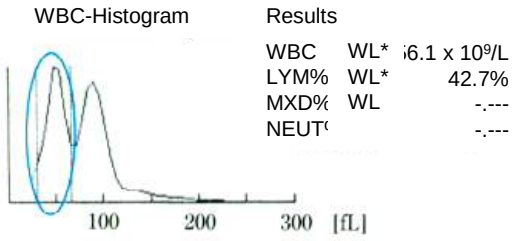


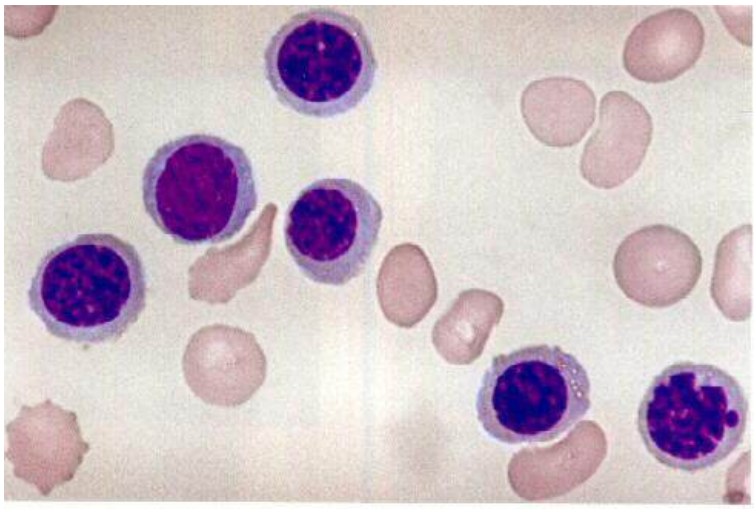
(x 1000)

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:

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

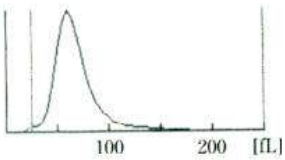
* NRBC Count: The number of NRBC per 100 leukocytes.

K-Series: Histogram Interpretation Cases

Anemia

Iron Deficiency Anaemia

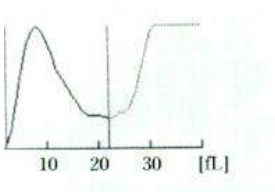
RBC-Histogram



Result

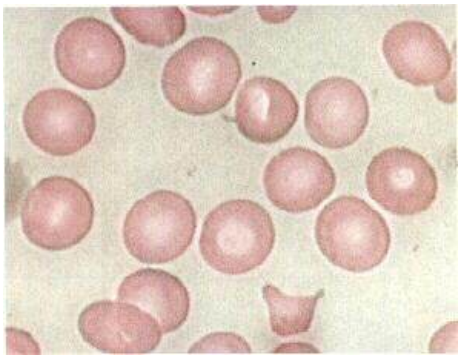
RBC		.48 x10^{12}/L
HGB		8.8g/dl
HCT		29.3%
MCV	–	65.4fl
MCH	–	19.6pg
MCHC	–	30.0g/dl
RDW-C	+	18.2%

PLT-Histogram



Result

PLT	PU*235 x10^{9}/L
PDW	11.7fl
MPV	9.4fl
P-LCR	21.7%



(x 1000)

1. Case:

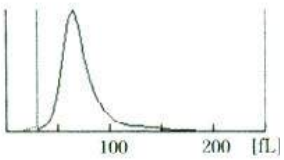
Results:
MCV, MCH and MCHC shows low values and RDW-SD shows a high value.

Differential:
hypochromic RBC´s

Thus this case is identified as microcytic hypochromic anemia

Suspected Thalassemia

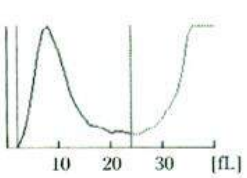
RBC-Histogram



Result

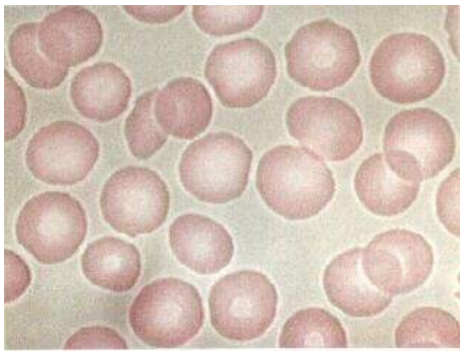
RBC	+	97 x10^{12}/L
HGB		12.7g/dl
HCT		41.1%
MCV	–	68.8fl
MCH	–	21.3pg
MCHC	–	30.9g/dl
RDW-		14.7%

PLT-Histogram



Result

PLT	391 x10^{9}/L
PDW	12.0fl
MPV	10.3fl
P-LCR	27.3%



(x 1000)

2. Case:

Results:
MCV, MCH and MCHC shows low values

Differential:
no prominence in the smear

Due to the increase in erythrocyte count and the low RDW value this case is cassified as a thalassaemia minor.

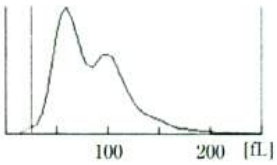
K-Series: Histogram Interpretation Cases

Anemia

Iron def. anaemia under treatment

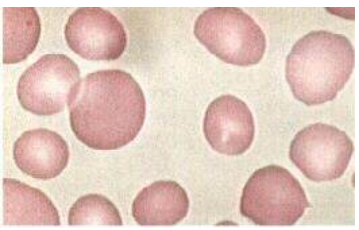
2nd Week of treatment

RBC-Histogram



Results

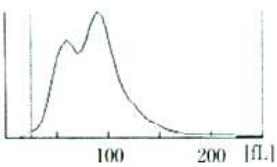
RBC		7 x10^{12}/L
HGB		10.4g/dl
HCT		35.3%
MCV	–	80.8fl
MCH	–	23.8pg
MCH	–	29.5g/dl
RDW	MP* +	35.7%



(x 1000)

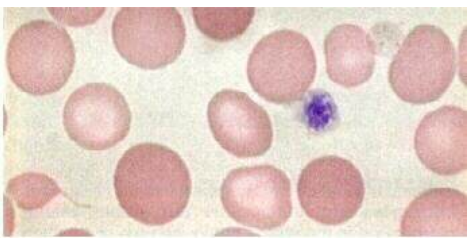
4nd week of treatment

RBC-Histogram



Results

RBC		4 x10^{12}/L
HGB		13.2g/dl
HCT		42.5%
MCV	–	82.7fl
MCH	–	25.7pg
MCH	–	31.1g/dl
RDW	MP* +	31.9%

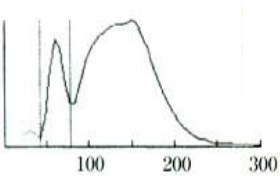


(x 1000)

The initial effect of the treatment can be seen in data of the 2th week, where the RBC histogram indicates the appearance of normocytic cells while a large number of microcytic cells still are visible in the smear. The RBC histogram of the 4th week still shows a 2-peak curve, but the peak of larger cells became more prominent than the other peak. Compared to the top diagram, this shows an further increase in the number of normocytes as a result of the treatment.

Macrocytic Anaemia (CML)

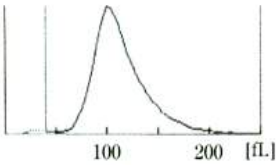
WBC-Histogram



Results

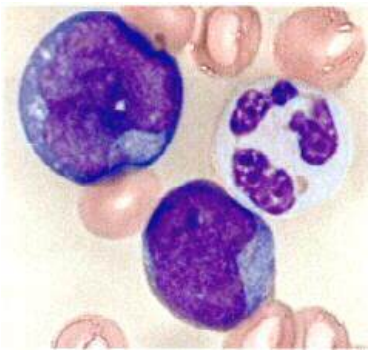
WBC	+	17.3 x10^{9}/L
LYM%		16.4%
MXD%	T2	-.---
NEUT %	T2	-.---

RBC-Histogram

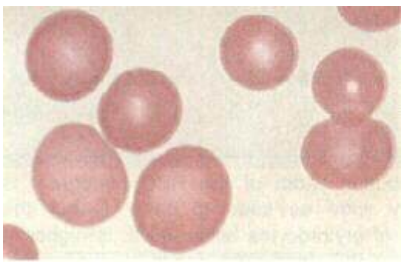


Results

RBC	–	..64 x10^{12}/L
HGB	–	6.2g/dl
HCT	–	18.2%
MCV	+	110.0fl
MCH		37.8pg
MCHC		34.1g/dl
RDW		15.2%



(x 1000)



(x 1000)

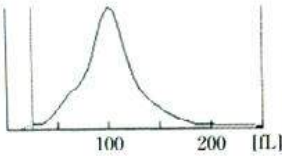
This is a macrocytic anaemia with development of chronic myelogenous leukemia (CML). The RBC histogram suggests the existence of macrocytes, while the WBC histogram does not show, the valley normally seen between the MXD and the large cell ratio, suggesting the appearance of leukocytes with various sizes.

K-Series: Histogram Interpretation Cases

Anisocytosis

Case1

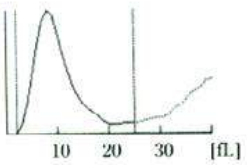
RBC-Histogram



Results

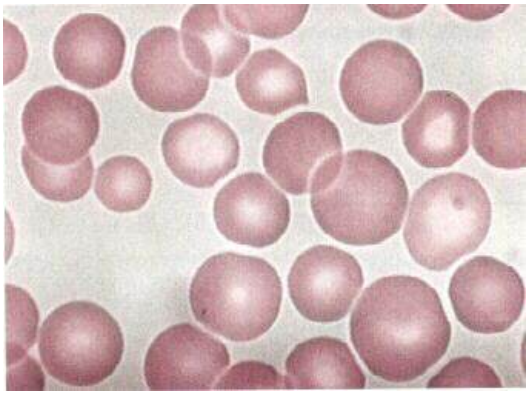
RBC		.15 x10^{12}/L
HGB		14.0g/dl
HCT		40.8%
MCV		98.3fl
MCH		33.7pg
MCHC		34.3g/dl
RDW	+	22.7%

PLT-Histogram



Results

PLT	328 x10^{9}/L
PDW	12.4fl
MPV	10.2fl
P-LCR	26.5%

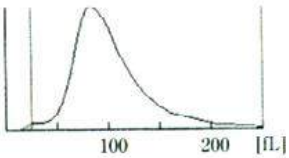


(x 1000)

Microcytes and macrocytes are visible among normocytes in the smear, and the distribution on the RBC histogram is abnormally wide. This suggest the appearance of various sizes of erythrocytes.

Case2

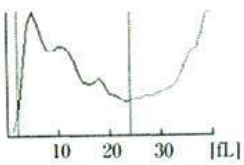
RBC-Histogram



Results

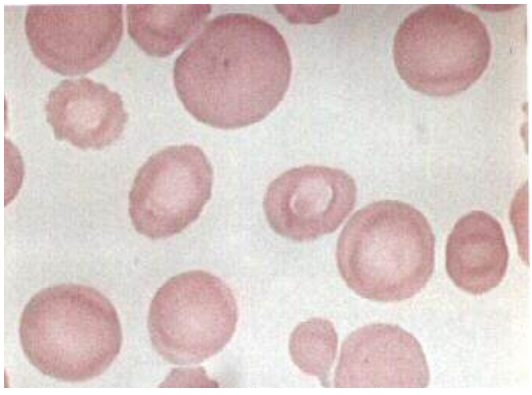
RBC		.95 x10^{12}/L
HGB		9.9g/dl
HCT		28.7%
MCV		97.3fl
MCH		33.6pg
MCHC		34.5g/dl
RDW	+	26.4%

PLT-Histogram



Results

PLT	PL'	98 x10^{9}/L
PDW	DW	---.-fl
MPV	PL	---.-fl
P-LCR	PL	-.---%



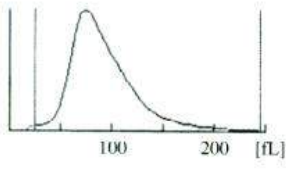
(x 1000)

The distribution width of the RBC histogram is abnormally wide as seen in case 1, but the proportion of erythrocytes below 90 fl is higher in case 2. The PLT histogram indicates abnormality and the PL and DW flags are given. This suggest that microcytes may have interferred with the Platelet count. Such result needs to be confirmed by other methods, like Fonio method or counting chamber.

Poikilocytosis

Case 1

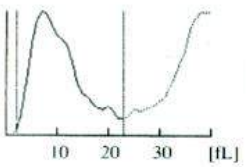
RBC-Histogram



Results

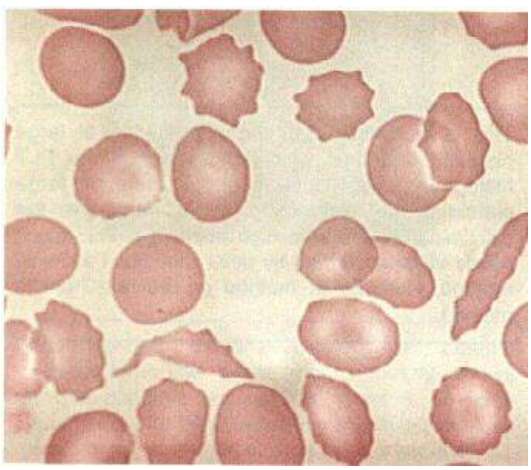
RBC		.62 x10^{12}/L
HGB		11.1g/dl
HCT		31.9%
MCV		88.1fl
MCH		30.7pg
MCHC		34.8g/dl
RDW	+	25.5%

PLT-Histogram



Results

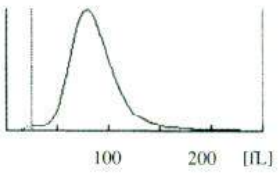
PLT	152 x10^{9}/L
PDW	16.2fl
MPV	10.4fl
P-LCR	31.4%



(x 1000)

Case 2

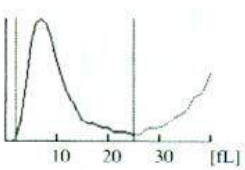
RBC-Histogram



Results

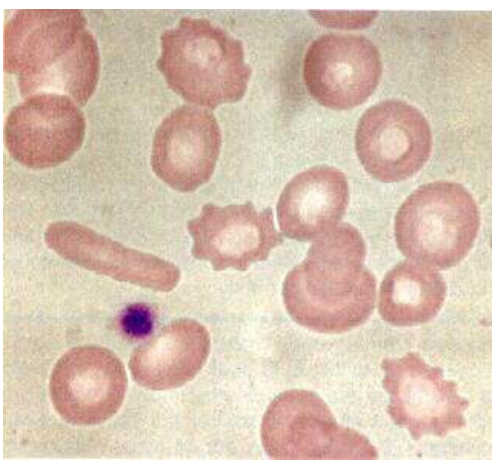
RBC		.67 x10^{12}/L
HGB	–	6.2g/dl
HCT	–	22.5%
MCV	–	84.3fl
MCH	–	23.2pg
MCHC	–	27.6g/dl
RDW	+	20.1%

PLT-Histogramm



Results

PLT	235 x10^{9}/L
PDW	11.7fl
MPV	9.4fl
P-LCR	21.7-%



(x 1000)

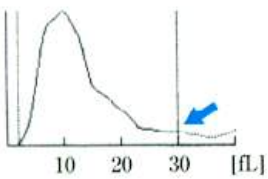
Two cases: Poikilocytosis with a lot of echinocytes

The abnormally wide distribution on the RBC histogram suggests the appearance of various sizes of erythrocytes with a high percentage of microcytes.

Large Platelets

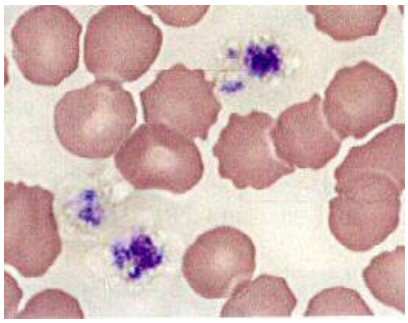
Case 1

PLT-Histogram



Results

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



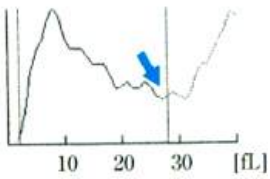
(x 1000)

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.

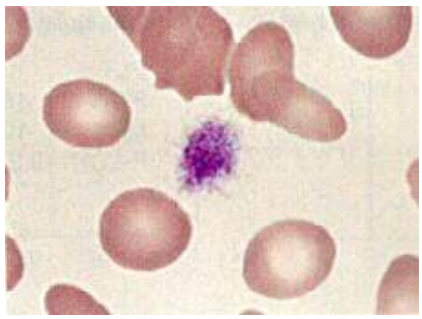
Case 2

PLT-Histogram



Results

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



(x 1000)

Case 2: large platelets

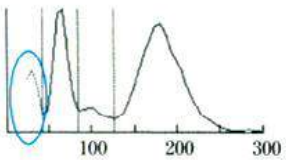
Although the wide distribution on the PLT histogram suggests the appearance of large platelets, the distribution curve intersects the discrimination line at a high point. This result needs to be confirmed by other methods i.e Fonio method or counting chamber.

K-Series: Histogram Interpretation Cases

Platelet Aggregation

Case 1

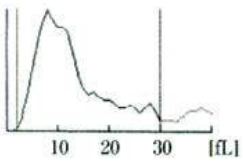
WBC-Histogram



Results

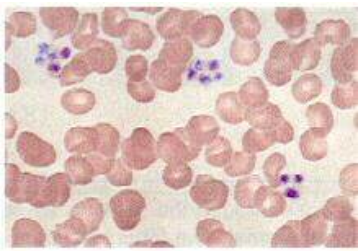
WBC		6.0 x10⁹/L
LYM%		27.5%
MXD%		7.9%
NEUT %		64.4%

PLT-Histogram



Results

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



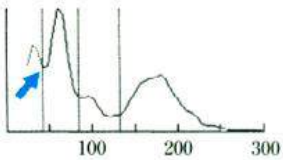
(x 400)

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

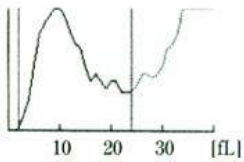
WBC-Histogram



Results

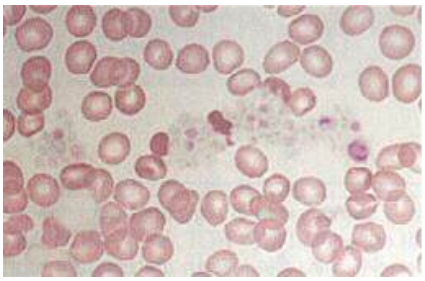
WBC	WL*	6.4 x10⁹/L
LYM%	WL*	41.4%
MXD%	WL*	14.0%
NEUT %	WL*	44.6%

PLT-Histogram



Results

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



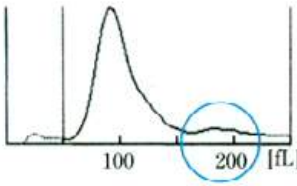
(x 400)

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.

Cold Agglutinins

RBC-Histogram

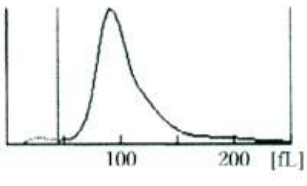


Results

RBC	RU*	2.23 x10^{12}/L
HGB		14.4g/dl
HCT	RU*	24.9%
MCV	RU*	111.7fl
MCH	RU*	64.6pg
MCHC	RU*	57.8g/dl
RDW	*	25.4fl

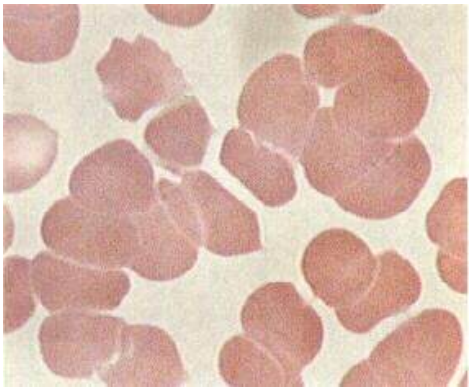
Incubation 30 min

RBC-Histogram

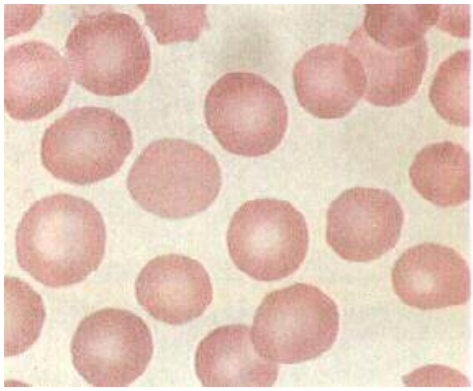


Results

RBC	4.35 x10^{12}/L
HGB	14.5g/dl
HCT	43.5%
MCV	100.0fl
MCH	33.3pg
MCHC	33.3g/dl
RDW	14.7fl



(x 1000)



(x 1000)

Case: Cold agglutinins

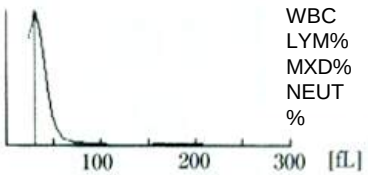
Because in this case erythrocytes have passed through the detector as clusters of several cells, the RBC, HCT,MCH, MCV, MCHC and RDW values are abnormal. The RBC histogram shows a second peak.

After the clusters have been dissolved by incubation, all erythrocytes are detected as single cells. Therefore the second peak on the RBC histogram does not appear and the RBC, HCT, MCV, MCH, MCHC and RDW values are normal.

K-Series: Histogram Interpretation

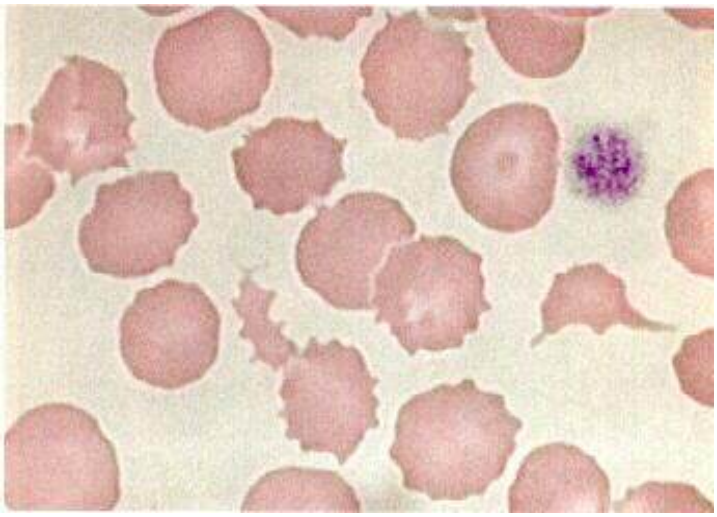
Insufficient Lysing of Erythrocytes

WBC-Histogram



Results

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



(x 1000)

Case: Lyse Resistance 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 solvedby diluting the sample or replacing plasma with cellpack (blood cell washing).

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